



# ACTA PHYSIOLOGICA SCANDINAVICA

VOL. 6



## REDACTORES

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1943

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Karolinska Institutet, Stockholm*

JOHNSON REPRINT CORPORATION    JOHNSON REPRINT COMPANY LIMITED  
111 Fifth Avenue, New York, N.Y. 10003    Berkeley Square House, London, W.1

First reprinting, 1964, Johnson Reprint Corporation

Printed in the United States of America

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From the State Institute of Public Health,  
Tomteboda, Sweden.

## Computation of Results from Experiments with Indirect Calorimetry.

By

ERNST ABRAMSON.

Received 2 March 1943.

For the computation of metabolism on the basis of data obtained in experiments with indirect calorimetry, J. E. JOHANSSON put forward in 1910 a mode of procedure which he developed on various occasions later though without carrying it to a conclusion at any time. Since JOHANSSON's death in 1938 I, who was formerly his assistant, have completed and extended this in various respects. This mode of computation, which may also be presented in the form of simple nomograms, facilitates in high degree the calculations necessary for such metabolism experiments.

### I. Respiratory Quotients and Calorific Values.

Normal metabolism in the body is taken as comprising three type substances, protein, fat and carbohydrate. The protein's nitrogen, together with parts of its carbon, hydrogen and oxygen, reappears in the urine, while the N-free remainder is converted along with the fat and carbohydrate into carbon dioxide and water by taking up oxygen. Just as protein, fat and carbohydrate are regarded in this connection as having a definite typical composition, this applies also to the composition and calorific value of organic substance in the urine. In computing the converted quantity of protein, the protein is taken as represented by its N-free

remainder, calculated per gram N in the urine. The combustion then comprises three substances, each of which represents c, h and o grams carbon, hydrogen and oxygen or  $n_C$  gram atoms of carbon,  $n_H$  and  $n_O$  gram molecules of hydrogen and oxygen as well as an energy amount of u calories calculated per gram metabolized substance or per gram N in metabolized protein. The carbon dioxide production will then be  $n_O$  gram molecules and the oxygen consumption  $\left(n_C + \frac{n_H}{2} - n_O\right)$  gram molecules. The substance's respiratory quotient will consequently be

$$q = \frac{n_O}{n_C + \frac{n_H}{2} - n_O}.$$

But  $n_C = \frac{c}{12.01}$ ,  $n_H = \frac{h}{2.016}$  and  $n_O = \frac{o}{32.00}$ .

Hence  $q = \frac{c}{c + 2.979 \cdot h - 0.3753 \cdot o}$ .

If the designation v is inserted for the oxygen consumption (litres per gram burned substance or litres per gram N in the urine) and  $w = \frac{u}{v}$  for the calorific value of 1 litre oxygen we get

$$v = \frac{22.41 \cdot n_C}{q} = 1.866 \frac{c}{q} (0^\circ C, 760 \text{ mm Hg}) \text{ and}$$

$$w = \frac{u \cdot q}{22.41 \cdot n_C} = 0.5359 \frac{u \cdot q}{c}.$$

These formulae may be employed for any substance burned in the body. Each substance is characterised by its numerical value for these magnitudes. But, besides the stated type substances, protein, fat and carbohydrate, other substances may be metabolized in the body, e. g. alcohol. In certain circumstances other final products than those mentioned may also arise, e. g.  $\beta$ -hydroxybutyric acid. In the event the composition and heat of combustion of such substances are known, it is possible to compute the numerical values of the constants q, v and w for them also. (Table 1.)

Table 1.

*Respiratory quotient (q), oxygen consumption (v), calorific value of 1 litre  $O_2$  (w) and heat of combustion (u) for some compounds.*

Compound	q	v	w	u
Protein <sup>1</sup>	0.802	5.976	4.491	26.84
Protein <sup>1a</sup>	0.801	5.939	4.464	26.51
Protein <sup>2</sup>	0.809	5.741	4.60	26.41
Fat <sup>1</sup>	0.707	2.020	4.686	9.461
Fat <sup>2a</sup>	0.711	2.013	4.72	9.50
Fat <sup>2b</sup>	0.713	1.992	4.79	9.54
Carbohydrate <sup>1</sup>	1.00	0.829	5.046	4.188
Carbohydrate <sup>2</sup>	1.00	0.829	5.06	4.20
Ethyl alcohol	0.667	1.459	4.87	7.11
$\beta$ -hydroxybutyric acid	0.889	0.969	4.84	4.69
Acetic acid	1.000	0.878	4.76	4.18
Acetone	0.750	1.544	4.76	7.35
Glucose	1.000	0.746	5.01	3.74
Pyruvic acid	1.200	0.636	4.99	3.17

## II. Computation of the Energy Metabolism.

Assume that the body's oxygen consumption for a certain period of time is  $O_2$  litres, its carbon dioxide production  $CO_2$  litres, its nitrogen excretion in the urine N grams and its energy metabolism U calories. Assume also that one or more other substances have been burned to carbon dioxide and water and that in the urine other metabolic products, such as  $\beta$ -hydroxybutyric acid, have been excreted.

Designate the shares of the three type substances, protein, fat and carbohydrate, in the oxygen consumption by  $x_p$ ,  $x_f$  and  $x_k$  and the shares of each of the other substances by  $x_1$ ,  $x_2$  etc. The oxygen consumption observed,  $O_2$ , will be equal to the sum of  $x_p$ ,  $x_f$  etc., it being noted that the terms  $x_1$ ,  $x_2$  and so on have positive signs when the substance in question is metabolized in

<sup>1</sup> After LUSK. (1928).

<sup>1a</sup> As in 1) but in this case when computing the N-free remainder deduction has not been made for the N-containing substance in the feces

<sup>2</sup> After BENEDICT (1924). As regards fat the values refer a) to animal fat, b) to human fat.

the body, and negative when it applies to a substance excreted in the urine. In the equation system below such a substance  $M$  is exemplified by the terms  $\pm x_m$ ,  $\pm q_m x_m$  and  $\pm w_m x_m$ . We may then put:

$$O_2 = x_p + x_t + x_k \pm x_m \dots \dots \dots \dots \dots \quad (1)$$

$$CO_2 = q_p x_p + q_f x_f + x_k \pm q_m x_m \dots \dots \dots \quad (2)$$

$$U = w_p x_p + w_f x_f + w_k x_k \pm w_m x_m \dots \dots \quad (3)$$

According to the definitions:

$$x_p = v_p \cdot N \quad \dots \dots \dots \dots \dots \dots \dots \dots \dots \quad (4)$$

From equations (1) and (2) we get:

$$x_f = \frac{1}{1 - q_f} [O_2 - CO_2 - v_p N (1 - q_p) \mp v_m M (1 - q_m)] \text{ and } (6)$$

$$x_k = \frac{1}{1 - q_t} [C_{O_2} - O_2 q_t - v_p N (q_p - q_t) \mp v_m M (q_m - q_t)] \quad (7)$$

After insertion of the above expressions for  $x_p$ ,  $x_f$ ,  $x_k$ , and  $x_m$  in equation (3) and introduction of suitable terms, we get:

$$U = d_u O_2 + e_u CO_2 + g_u N \pm h_u M \quad \dots \dots \dots \quad (8)$$

where

$$d_u = \frac{w_f - w_k q_f}{1 - q_f},$$

$$e_u = \frac{w_k - w_f}{1 - q_f};$$

$$g_u = v_p \left[ w_p - \frac{w_f (1 - q_p)}{1 - q_t} - \frac{w_k (q_p - q_f)}{1 - q_t} \right] \text{ and}$$

$$h_u = \pm v_m \left[ w_m - \frac{w_f(1-q_m)}{1-q_f} - \frac{w_k(q_m-q_f)}{1-q_f} \right].$$

### III. Computation of the Metabolized Amounts of Protein, Fat and Carbohydrate.

a) *Protein.* The type substance protein is assumed to contain 0.1665 g nitrogen per gram. We thus get the metabolized amount of protein from the amount of nitrogen observed in the urine by

multiplying by the factor 6.00. If account is taken of the fact that 0.0037 g of the protein's nitrogen is to be found in the feces, the corresponding value for the factor will be 6.14.

b) *Fat.* The fat metabolism, F, is obtained from equation (6) by dividing by  $v_f$ . After introduction of similar designations as in equation (8), we get

$$F = d_f O_2 + e_f CO_2 + g_f N \pm h_f M, \dots \dots \dots \quad (9)$$

where

$$d_f = -e_f = \frac{1}{v_f(1-q_f)}; g_f = -\frac{v_p(1-q_p)}{v_f(1-q_f)}; h_f = \mp \frac{v_m(1-q_m)}{v_f(1-q_f)}.$$

c) *Carbohydrate.* Correspondingly the carbohydrate metabolism, K, is obtained from equation (7) by dividing by  $v_k$ . After introduction of similar terms as in equation (8), we get

$$K = d_k O_2 + e_k CO_2 + g_k N \pm h_k M, \dots \dots \dots \quad (10)$$

where

$$d_k = -\frac{q_f}{v_k(1-q_f)}; e_k = \frac{1}{v_k(1-q_f)}; g_k = -\frac{v_p(q_p-q_f)}{v_k(1-q_f)} \text{ and}$$

$$h_k = \mp \frac{v_m(q_m-q_f)}{v_k(1-q_f)}.$$

d) *Some simple relations between the coefficients d, e and g.*

Between the coefficients d, e and g there exist certain simple relations, as may be seen from the summary below.

$$d_u + e_u = w_k$$

$$d_u + q_f e_u = w_f$$

$$d_u + q_p e_u + \frac{g_u}{v_p} = w_p$$

$$d_f + e_f = 0$$

$$d_k + e_k = \frac{1}{v_k} = e_k(1-q_f)$$

$$d_f(1-q_f) = \frac{1}{v_f}$$

These formulae may likewise be employed for checking the computed numerical values of the coefficients d, e and g.

#### IV. Transformation of one Type Substance into another.

From equations (6) and (7) and from (9) and (10) there is seen the possibility that the calculated fat or carbohydrate metabolism may have a negative value. It is most simple to interpret a "negative combustion" as a new formation. It is, however, easy to realise that it cannot be question of a process in the opposite direction to combustion, the oxidation process. The initial material for such a process would, of course, be the carbon dioxide and the water given off by the body during the period of observation. It can therefore only be question of a displacement of the amounts of the type substances among themselves, an increase in the quantity of fat or carbohydrate at the expense of the others. The result of such a transformation process may appear at the termination of the observation period as a store in the body, as an excretion product (e. g. sugar) in the urine or merely as a reduction in the customary fat or carbohydrate metabolism. In the two first cases the metabolism, computed according to (9) and (10), is negative, in the last case positive. The formulae in question give therefore the sum of the results of the combustion and of any transformation processes.

Our deductions, however, have not assumed any other processes than those that can be designated as protein, fat or carbohydrate combustion and it is therefore necessary to go further into the transformation processes to which the expressions (8), (9) and (10) are applicable.

a) *Fat formation from carbohydrate.* Assume to begin with that a certain amount of carbohydrate,  $y$  grams, is transformed into 1 gram fat, developing carbon dioxide and water but not taking up any oxygen. Complete combustion of this amount of carbohydrate would obviously mean the same oxygen consumption as 1 gram fat. Then, according to the definitions under I

$$v_f = y \cdot v_k, \text{ from which}$$

$$y = \frac{v_f}{v_k} = \frac{n_{C(t)}}{q_f \cdot n_{C(k)}}.$$

As  $y$  grams carbohydrate contain  $y \cdot n_{C(k)} = \frac{n_{C(t)}}{q_f}$  gram atoms carbon, the amount of carbon in the carbon dioxide formed by this conversion is  $\left[ \frac{n_{C(t)}}{q_f} - n_{C(t)} \right]$  gram atoms. The litre value of the amount of carbon dioxide in question is equal to  $22.41 \left[ \frac{n_{C(t)}}{q_f} - n_{C(t)} \right]$ , which expression may be written  $v_f(1 - q_f)$ . The same amount of carbohydrate gives off on complete combustion  $\frac{u_k \cdot v_f}{v_k}$  calories. In the transformation

described therefore there is liberated  $\frac{u_k \cdot v_f}{v_k} - u_f = v_f (w_k - w_f)$  calories in the form of heat. The amount of energy liberated per litre of carbon dioxide will thus be  $\frac{w_k - w_f}{1 - q_f}$ . This expression is, as may be seen, identical with the coefficient  $e_u$  in equation (8). The fat formation per litre of carbon dioxide formed is  $\frac{1}{v_f (1 - q_f)}$  which value agrees numerically with the value of the coefficient  $e_f$  in equation (9), but is opposite in sign, which of course, in accordance with the above and as should be the case, indicates that it is not a matter of fat combustion but an augmentation of the fat store at the expense of that of the carbohydrate. The quantity of carbohydrate burned per litre of carbon dioxide is  $\frac{1}{v_k (1 - q_f)}$  or equal to the coefficient  $e_k$  in equation (10).

b) *Carbohydrate formation from fat.* If it is instead a matter of a transformation of fat to carbohydrate it should be noted that  $\frac{n_{C(f)}}{n_{C(k)}}$  grams fat contain the same amount of carbon as 1 gram carbohydrate. In complete combustion carbohydrate requires no taking up of oxygen beyond that corresponding to the amount of carbon. Fat on the other hand requires a further  $\left[ \frac{n_{H(f)}}{2} - n_{O(f)} \right]$  gram molecules oxygen per gram substance. A carbohydrate formation from fat, as defined here, is thus characterised by an oxygen consumption of  $\frac{n_{C(f)}}{n_{C(k)}} \cdot \left[ \frac{n_{H(f)}}{2} - n_{O(f)} \right] = \frac{n_{C(f)}}{q_f} (1 - q_f)$  gram molecules per gram of carbohydrate formed, equivalent to  $\frac{v_k}{q_f} (1 - q_f)$  litres oxygen per gram carbohydrate formed. The amount of energy liberated, calories per gram carbohydrate formed, is  $u_f \cdot \frac{n_{C(f)}}{n_{C(k)}} - u_k = v_k \left( \frac{w_f}{q_f} - w_k \right)$ . The amount of energy liberated per litre oxygen consumed will then be  $\frac{w_f - w_k \cdot q_f}{1 - q_f}$ .

This expression is identical with the coefficient  $d_u$  in equation (8). The carbohydrate formation per litre oxygen consumed is  $\frac{q_f}{v_k (1 - q_f)}$  which value agrees numerically with the value of the coefficient  $d_k$  in equation (10), but has the opposite sign. The quantity of fat burned per litre oxygen is  $\frac{1}{v_f (1 - q_f)} = d_f$  in equation (9).

It has therefore appeared that the energy metabolism calories per litre  $CO_2$  in the transformation of carbohydrate to fat, and calories per litre  $O_2$  in the conversion of fat to carbohydrate, has the same value as the coefficient  $e_u$  and  $d_u$  respectively in equation (8). In the same way the expressions for fat formation per litre carbon dioxide produced and for carbohydrate formation per litre oxygen consumption agree with

the numerical values for the coefficients  $e_k$  or  $d_k$  for  $\text{CO}_2$  and  $\text{O}_2$  respectively in equations (9) and (10), which give expression to the change in the body's fat and carbohydrate amounts respectively. Finally, the expressions for the carbohydrate quantity metabolized per litre  $\text{CO}_2$  and the fat quantity per litre oxygen consumption also agree with the values for  $e_k$  and  $d_k$  respectively in the equations (10) and (9). If, therefore, a certain portion of the carbon dioxide production or the oxygen consumption should come from a fat formation from carbohydrate or carbohydrate formation from fat, yet irrespective of this the energy metabolism of the body as also the changes in the fat and carbohydrate amounts may be computed according to equation (8) or (9) and (10).

c) *Carbohydrate formation from protein.* The same applies in the event a part of the oxygen consumption or carbon dioxide production comes from a carbohydrate or fat formation from protein, from which process a portion of the nitrogen excretion by the urine likewise has its origin in such a case. Indeed, if the reasoning just presented taking the protein's nitrogen-free remainder as basic material is applied, it is evident that for a carbohydrate formation from protein  $\frac{n_{C(k)}}{n_{C(p)}} = \frac{v_k}{v_p \cdot q_p}$  grams protein contains the same amount of carbon as 1 gram carbohydrate. The requisite oxygen consumption in litres will then be  $22.41 \frac{n_{C(k)}}{n_{C(p)}} \left[ \frac{n_{H(p)}}{2} - n_{O(p)} \right] = \frac{v_k}{q_p} (1 - q_p)$  per gram carbohydrate formed or  $v_p (1 - q_p)$  litres oxygen per gram N. The energy production is obviously  $u_p \frac{n_{C(k)}}{n_{C(p)}} - u_k = v_k \left( \frac{w_p}{q_p} - w_k \right)$  calories per gram carbohydrate formed, equivalent to  $\frac{w_p - w_k \cdot q_p}{1 - q_p}$  calories per litre oxygen or  $v_p (w_p - w_k q_p)$  calories per gram excreted nitrogen.

Let  $O'_2$  and  $N'$  express that part of the oxygen consumption or nitrogen excretion respectively that corresponds to the transformation and  $U'$  the corresponding share in the body's energy production. No part of the carbon dioxide production belongs to the transformation process itself, which, naturally, does not exclude that the transformation may be combined with a protein combustion in the ordinary manner, i. e. that already considered on the basis of equation (8).

That equation should for the case in question therefore be written  $U' = d_u O'_2 + g_u N'$ . If there be inserted in this equation the values for  $d_u$  and  $g_u$  from equation (8), and the above deduction for oxygen consumption per gram nitrogen, we get  $U' = v_p (w_p - w_k q_p) N$ , which expression is identical with that just given for energy production in the process concerned.

d) *Fat formation from protein.* A similar procedure may be employed for computing the conditions of a fat formation from protein as in computing fat formation from carbohydrate. If we consider  $z$  gram protein transformed into 1 gram fat, then  $z = \frac{v_f}{v_p} = \frac{n_{C(f)} \cdot q_p}{n_{C(p)} \cdot q_f}$ . The

litre value of the carbon dioxide given off in the transformation of  $z$  gram protein is therefore  $22.41 \frac{n_{C(f)} \cdot q_p}{q_f} - n_{C(f)} = v_f (q_p - q_f)$  litres  $\text{CO}_2$  per gram fat formation equivalent to  $v_p (q_p - q_f)$  litres  $\text{CO}_2$  per gram N. The energy produced will be  $\frac{u_p \cdot v_f}{v_p} - u_f = v_f (w_p - w_f)$  calories per gram fat formation corresponding to  $\frac{w_p - w_f}{q_p - q_f}$  calories per litre carbon dioxide formed or  $v_p (w_p - w_f)$  calories per gram N.

As  $w_f > w_p$  the part,  $U'$ , of the process in the body's energy conversion is negative, i. e., the process must be regarded as being coupled with some partial process entering into the assumed combustion processes, by which energy is made available. However, we have in analogy with the preceding case,  $U' = e\text{CO}'_2 + g\text{N}'$ , from which it follows that, even if during a period of observation the fat amount of the body should have increased at the expense of the protein amount yet the body's energy production may irrespective of this be computed according to the formula (8).

Indirect calorimetry, as already stated, gives no information about the chemical nature of the transformation processes in question. When there is taken into account the share, for example, the transformation process protein to carbohydrate or fat has in the values noted for the body's oxygen consumption, carbon dioxide production and nitrogen excretion, the possibility is not excluded that the transformation process may be combined with a combustion of greater or less extent. By assuming in the computation a production of carbon dioxide and water without oxygen consumption or an oxygen consumption above that corresponding to the carbon quantity in the basic material, the transformation processes as well as the combustion processes will be defined. As pointed out above, the influence of the transformation processes is opposite to that of the combustion processes in respect of the fat or carbohydrate metabolism expressed in equations (9) and (10). In conformity with this the share of the stated transformation processes in the fat or carbohydrate metabolism, amounting as above to  $\frac{v_p}{v_f} N'$  or

$\frac{v_p \cdot q_p}{v_k} N'$ , must be regarded as negative. The same expressions are obtained if the equations (9) and (10) are based on, taking into account that the transformation process protein to fat or carbohydrate has no share in the body's oxygen consumption or carbon dioxide production and, if the expressions,  $\text{CO}'_2 = v_p (q_p - q_f) N'$  or  $\text{O}'_2 = v_p (1 - q_p) N'$ , are introduced as was done above. It is therefore obvious that a computation in accordance with formula (8) can be applied without further alteration even if the values observed for the body's oxygen consumption, carbon dioxide production and nitrogen excretion in greater or less degree originate from processes in which a transformation of one type substance to another has taken place.

## V. The Body's Respiratory Quotient.

If the volume of carbon dioxide given off during the observation period is divided by the volume of oxygen produced during the same time, there is obtained the body's respiratory quotient,  $Q$ . Employing the formulae (8), (9) and (10), we get:

$$U = \frac{w_f - w_k \cdot q_f + Q (w_k - w_f)}{1 - q_f} O_2 + g_u N \dots \quad (8 \text{ a})$$

$$F = \frac{1 - Q}{v_f (1 - q_f)} O_2 + g_f N \dots \dots \dots \dots \dots \quad (9 \text{ a})$$

$$K = \frac{Q - q_f}{v_k (1 - q_f)} O_2 + g_k N \dots \dots \dots \dots \dots \quad (10 \text{ a})$$

If the actual respiratory quotient has the value  $q_p$  — the respiratory quotient for the type substance protein — then there occurs no other change in the appearance of the stated formulae than that  $Q$  is substituted by  $q_p$  throughout. If, however,  $Q$  has the value  $q_f$  or 1 — the respiratory quotient for fat or carbohydrate — they assume a simpler form.

For  $Q = q_f$  we get

$$U = w_f O_2 + g_u N$$

$$F = \frac{1}{v_f} O_2 + g_f N$$

$$K = g_k N,$$

and for  $Q = 1$ ,

$$U = w_k O_2 + g_u N$$

$$F = g_f N$$

$$K = \frac{1}{v_k} O_2 + g_k N.$$

A purely protein combustion takes place when  $F = K = 0$ , i. e. when

$$\frac{v_k (1 - Q)}{v_f (Q - q_f)} = \frac{g_f}{g_k}.$$

By substituting in this expression the values for  $g_f$  in equation (9) and for  $g_k$  in equation (10), we get  $Q = q_p$ . The condition for a purely protein combustion occurring is therefore that  $Q = q_p$ .

On the other hand, a respiratory quotient  $Q = q_p$  does not necessarily mean that a purely protein combustion takes place.

A purely carbohydrate or fat combustion cannot occur without a simultaneous protein combustion. The formulae make it clear that with an observed respiratory quotient of 1, the magnitude of the metabolism is not obtained by multiplying the amount of oxygen consumed by the calorific value of the oxygen for carbohydrate  $w_k$ . In actual fact the metabolism is somewhat smaller, dependent on the magnitude of the protein metabolism occurring. The amount of carbohydrate converted is then rather smaller than that corresponding to the oxygen consumption multiplied by  $\frac{1}{v_k}$ .

When  $Q = 1$  there takes place a displacement of the body's store of substance to the benefit of fat. If it is assumed that for basal metabolism the body's oxygen consumption has in one case been measured as 14 litres per hour and the amount of nitrogen in the urine as 0.25 grams, it will be found that the carbohydrate metabolism will be zero for a respiratory quotient  $Q = 0.717$ . As  $q_f$  is 0.707 the difference due to the simultaneous protein metabolism is therefore extremely small. A displacement to the advantage of the carbohydrate store, i. e. so that  $K$  in equation (10) holds a negative value, occurs therefore in this case immediately  $Q$  becomes less than 0.717.

In principle the circumstances are similar for a respiratory quotient  $Q = q_f$ . There then occurs an augmentation of the body's carbohydrate store at the expense of the other type substances. With the values assumed above for oxygen consumption and amount of nitrogen in the urine we get  $K = 0$  with  $Q = 0.98$ . If in this case the respiratory quotient exceeds 0.98 then  $K$  will be negative.

## VI. Numerical Values of the Different Coefficients. Nomograms.

The numerical values for the coefficients  $d$ ,  $e$  and  $g$  have, by utilisation of the values given in table 1 for  $q$ ,  $v$  and  $w$  for protein, fat and carbohydrate, been presented in table 2.

It will be seen that the values for  $d$ ,  $e$  and  $g$ , calculated according to the various assumptions regarding the composition of the type substances, vary but little one from the other. The greatest is the difference in regard to coefficient  $g_u$ . This, however, in practice

is rather insignificant, as this term plays numerically a relatively subordinate rôle in the computation of the magnitude of the energy metabolism. The preponderating part of the protein's combustion value is already to be found in the coefficients  $d_u$  and  $e_w$ , referring to oxygen and carbon dioxide. If it be assumed that the total metabolism concerns protein, obviously  $U$  would be equal to  $u_p \cdot N$ . The error arising if the term  $g_u$  were left out in computing the energy metabolism according to equation (8) would therefore at the outside be equal to  $\frac{g_u}{u_p} = -0.075$ . Thus the error amounts at most to only 7.5 %, i. e. we get with purely protein combustion a value 7.5 % too high, if the term  $g_u N$  is neglected. Generally, however, the error is appreciably less. As protein combustion as a rule represents less than 15 % of the total metabolism, the error is therefore usually about 1 %.

Table 2.  
Numerical values for  $d$ ,  $e$  and  $g$ .

	$d_u$	$e_u$	$g_u$	$d_f$	$e_f$	$g_f$	$d_k$	$e_k$	$g_k$
1.	3.82	1.23	- 2.00	1.69	- 1.69	- 2.00	- 2.91	4.12	- 2.30
1a.	3.81	1.24	- 1.85	1.69	- 1.69	- 2.00	- 2.91	4.12	- 2.34
2a.	4.11	0.94	- 1.61	1.72	- 1.72	- 1.88	- 2.97	4.17	- 2.35
2b.	3.88	1.18	- 1.38	1.74	- 1.74	- 1.90	- 3.00	4.20	- 2.82

If we insert in table 2 the values given for the different coefficients in the equations stated earlier, they would have for normal metabolism the following appearance.

Energy metabolism, calories  $U = 3.82 O_2 + 1.23 CO_2 - 2.00 N$ .  
Protein, grams . . . . .  $P = 6.14 N$ .

Fat, grams . . . . . F = 1.69 O<sub>2</sub> — 1.69 CO<sub>2</sub> — 2.00 N.

Carbohydrate, grams . . . K = 4.12 O<sub>2</sub> - 2.91 CO<sub>2</sub> - 2.30 N.

The shares of the different type substances in the oxygen con-

The shares of the different type substances in the oxygen consumption amount to, for:

fat . . . . . 3.413 ( $O_2 - CO_2 - 1.182 N$ )

carbohydrate . . . . . 3.413 ( $\text{CO}_2$  — 0.707  $\text{O}_2$  — 0.5583 N).

It is easy to see that the sum of the three last-named quantities is, as it should be, equal to  $O_2$ . The shares of the different type substances in the calory metabolism is obtained by multiplying the converted quantities computed above by their respective values of heat of combustion,  $u$ . (Table 1).

With the aid of the above equations the computation of a metabolism experiment is executed surely and rapidly. It can be still further facilitated if, after computing the respiratory quotient  $CO_2/O_2$  and the ration  $N/O_2$ , one or both of the attached nomograms is employed.

*Nomogram A* gives directly the figure by which the amount of oxygen consumed is to be multiplied in order to arrive at the total energy metabolism (the oxygen's calorific value), and the amounts of fat and carbohydrate burned. The amount of protein is obtained, as stated, by multiplying the amount of nitrogen found by 6.14. From *nomogram B* we obtain the percentage of share of the three type substances in the total combustion and in the oxygen consumption. The calorific value of the oxygen in various respiratory quotients, where the protein metabolism amounts to 10, 15, 20 or 25 % of the total metabolism has, moreover, been given in table 3.

*Example.*

Name: L. S.	Date: 12th December 1941. Exp. No. 201.
Urinary nitrogen	0.385 g/hour
$CO_2$ production	22.36 g/hour
$O_2$ consumption	20.04 g/hour

Converting grams  $CO_2$  and  $O_2$  to litres.

$$22.36 \times 0.5089 = 11.38 \text{ litres } CO_2.$$

$$20.04 \times 0.6997 = 14.02 \text{ litres } O_2.$$

$$R. Q. = 0.812.$$

$$N/O_2 = 0.0275.$$

$$\text{Protein} = 6.14 \times 0.385 = 2.36 \text{ grams.}$$

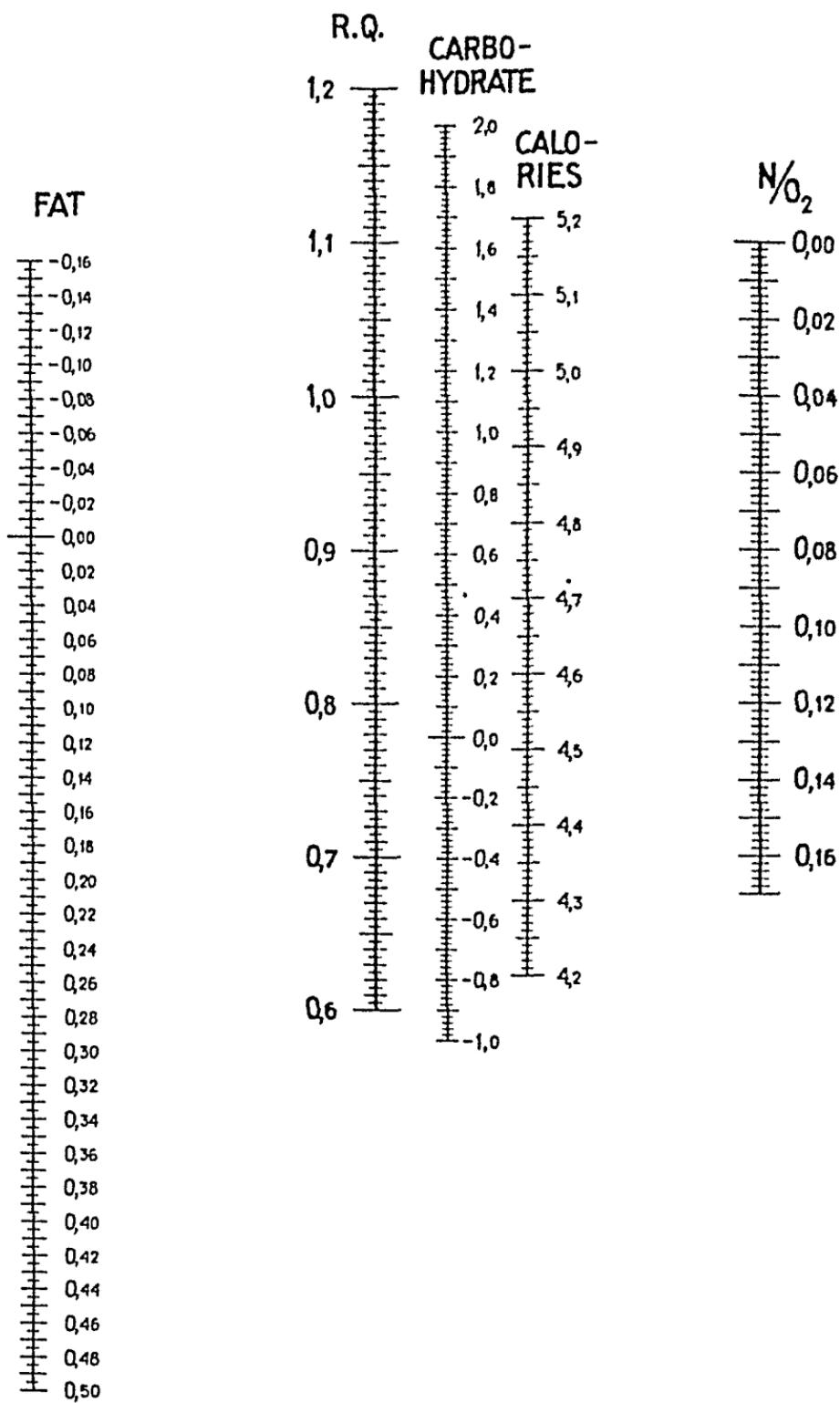
From monogram A we get the figures 4.76, 0.263 and 0.371, by which the amount of oxygen consumed has to be multiplied in order to arrive at the energy, fat and carbohydrate metabolism respectively.

$$\text{Calories} = 14.02 \times 4.76 = 66.7 \text{ calories.}$$

$$\text{Fat} = 14.02 \times 0.263 = 3.69 \text{ grams.}$$

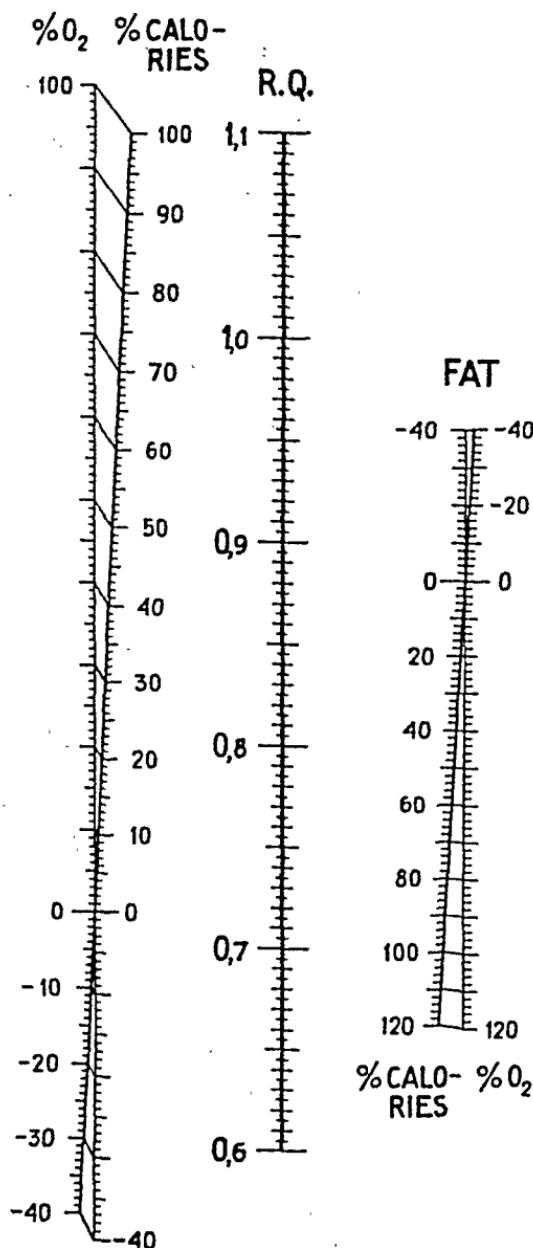
$$\text{Carbohydrate} = 14.02 \times 0.371 = 5.20 \text{ grams.}$$

By means of nomogram B we find that of the total calories 15.3 % come from protein, 52.4 % from fat and 32.3 % from carbohydrate. As regards the oxygen 16.3 % comes from the protein combustion, 53.1 % from fat and 30.6 % from carbohydrate.

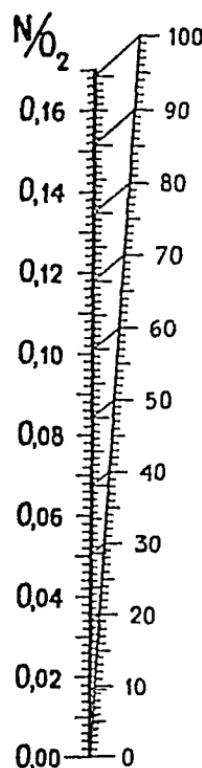
*Nomogram A*

for calculating the energy metabolism and the amount of fat and carbohydrate burned.

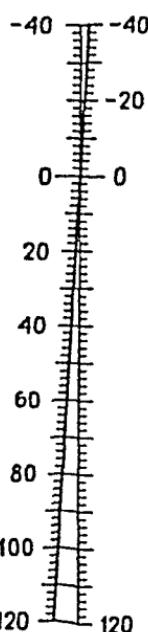
## CARBOHYDRATE



## PROTEIN

%O<sub>2</sub> %CALORIES

## FAT

% CALORIES % O<sub>2</sub>

Nomogram B

for calculating the percentage of share of protein, fat and carbohydrate in the total combustion and in the oxygen consumption.

Table 3.

*Calorific values of oxygen for different values of the respiratory quotient and for various levels of protein metabolism.*

R.Q.	Protein metabolism in % of the total energy metabolism			
	10 %	15 %	20 %	25 %
1.00	5.01	5.00	4.98	4.96
0.99	5.00	4.98	4.97	4.95
0.98	4.99	4.97	4.95	4.94
0.97	4.98	4.96	4.94	4.92
0.96	4.96	4.95	4.93	4.91
0.95	4.95	4.93	4.92	4.90
0.94	4.94	4.92	4.90	4.89
0.93	4.93	4.91	4.89	4.88
0.92	4.91	4.90	4.88	4.86
0.91	4.90	4.88	4.87	4.85
0.90	4.89	4.87	4.86	4.84
0.89	4.88	4.86	4.84	4.83
0.88	4.87	4.85	4.83	4.81
0.87	4.85	4.84	4.82	4.80
0.86	4.84	4.82	4.81	4.79
0.85	4.83	4.81	4.79	4.78
0.84	4.82	4.80	4.78	4.76
0.83	4.80	4.79	4.77	4.75
0.82	4.79	4.77	4.76	4.74
0.81	4.78	4.76	4.74	4.73
0.80	4.77	4.75	4.73	4.72
0.79	4.76	4.74	4.72	4.70
0.78	4.74	4.73	4.71	4.69
0.77	4.73	4.71	4.70	4.68
0.76	4.72	4.70	4.68	4.67
0.75	4.71	4.69	4.67	4.65
0.74	4.69	4.68	4.66	4.64
0.73	4.68	4.66	4.65	4.63
0.72	4.67	4.65	4.63	4.62
0.71	4.66	4.64	4.62	4.61
0.70	4.64	4.63	4.61	4.59
0.69	4.63	4.61	4.60	4.58
0.68	4.62	4.60	4.59	4.57
0.67	4.61	4.59	4.57	4.56
0.66	4.59	4.58	4.56	4.54
0.65	4.58	4.57	4.55	4.53

## VII. Computation of Metabolism when Substances other than Protein, Fat and Carbohydrate are Burned or when Foreign Matters Occur in the Urine.

In the discussion up to now the conversion of substances other than the type substances protein, fat and carbohydrate has not been closely considered. There can, however, be included in equations (1), (2) and (3) a number of terms which are there grouped under the designation  $\pm x_m$ ,  $\pm q_m x_m$  and  $\pm w_m x_m$ , and which refer to the occurrence of substances other than the above three type substances. The coefficients  $d$ ,  $e$  and  $g$  occurring in equations (8), (9) and (10) are only affected as regards their numerical magnitude by the assumed composition of the type substances, but not at all by other substances. A computation of metabolism when other substances are burned or when foreign matters occur in the urine is shown only by the appearance in these last named equations of a number of terms  $h$ , the numerical values of which are dependent on the chemical composition and heat of combustion of the substances in question.

Assuming that among the values observed there is included  $M'$  grams of such a foreign matter, which during the experimental period has been burned in the body into carbon dioxide and water, there would then appear a fourth term in the equations (8), (9) and (10) containing  $M'$  with the coefficients  $h'_u$ ,  $h'_f$  and  $h'_k$ . The introduction of  $M'$  and the values for the  $h'$ -coefficients derived from its chemical composition is naturally applicable to any substance whatever that is administered to the body and then stored in or excreted from it. There is therefore nothing to prevent the assumption that the substance  $M'$  constitutes one of the body substances protein, fat or carbohydrate. In such conditions the calculation will comprise what has been described above as a displacement among the body substances. It is easy to see that, if  $M'$  is taken as being fat,  $h'_u$  and  $h'_k$  will be equal to zero. On the other hand we have  $h'_f = -1$ , which demonstrates that a storage (or possibly excretion) of fat has taken place. Similarly if  $M'$  is taken as being carbohydrate,  $h'_u$  and  $h'_f$  will be equal to zero while  $h'_k$  has the value  $-1$ . If  $M'$  is taken as protein there will be a nitrogen excretion in the urine. If the computation be made with this as basis it will be found that the  $h'$ -coefficients become identical with the equivalent  $g$ -coefficients.

Finally, if it is assumed that during the period of observation there occur final products in the urine differing from the normal e. g.  $\beta$ -hydroxybutyric acid, we still start with the equations (1), (2) and (3) containing respectively the terms  $-x''_m$ ,  $-q''_m x''_m$  and  $-w''_m x''_m$ , where  $-x''_m = v_m M''$ . Let  $M''$  designate, say,  $\beta$ -hydroxybutyric acid excreted during the observation period and  $x_p$ ,  $x_f$  and  $x_k$  the shares of the three type substances in the oxygen consumption which would have taken place had combustion been complete, i. e. proceeded without formation of  $\beta$ -hydroxybutyric acid. In the formulae (8), (9) and (10) this is apparent through the occurrence of the terms containing  $M''$  with the coefficients  $h''_u$ ,  $h''_f$  and  $h''_k$ . The introduction of  $M''$  may naturally refer also to the type substances themselves. In such conditions, if  $M''$  is taken to be fat, then  $h''_u$  and  $h''_k$  will be equal to zero, while  $h''_f = 1$ . If instead  $M''$  is assumed to be carbohydrate then  $h''_u$  and  $h''_f$  will be equal to zero, while  $h''_k$  will equal 1. If  $M''$  is taken as nitrogen arising after protein combustion it will be found that the  $h''$ -coefficients will be constructed identically with the equivalent  $g$ -coefficients.

Table 4.

*The numerical values of the constants  $h_u$ ,  $h_f$  and  $h_k$  for certain compounds.*

Compound	$h_u$	$h_f$	$h_k$
Ethyl alcohol . . . . .	0.84	0.82	- 0.24
$\beta$ -hydroxybutyric acid . . .	- 0.07	0.18	0.78
Acetic acid . . . . .	- 0.25	0.00	1.06
Acetone . . . . .	- 0.04	0.65	0.27
Glucose . . . . .	- 0.08	0.00	0.90
Pyruvic acid . . . . .	- 0.20	- 0.22	1.29

These considerations respecting substances represented by  $M'$  and  $M''$  are therefore conclusive concerning the utility of equations (8), (9) and (10) for computation of energy and type substance metabolism in the body under various conditions. Finally in table 4 there are presented the numerical values of the coefficients  $h_u$ ,  $h_f$  and  $h_k$  for different compounds of the kind. When inserting these values in the respective formulae care should be taken to observe the signs preceding them in the equations in each indi-

vidual case. Application of what is put forward here may be illustrated by giving the necessary equations in computing a case where, in addition to protein, fat and carbohydrate, there is metabolized in the body during the observation period A grams alcohol and excreted B grams  $\beta$ -hydroxybutyric acid together with S grams glucose.

$$U = 3.82 O_2 - 1.23 CO_2 - 2.00 N + 0.34 A + 0.07 B + 0.03 S.$$

$$P = 6.14 N.$$

$$F = 1.69 O_2 - 1.69 CO_2 - 2.00 N - 0.82 A + 0.18 B.$$

$$K = 4.12 CO_2 - 2.91 O_2 - 2.30 N + 0.24 A + 0.73 B + 0.90 S.$$

### Summary.

A simple method for computing results from experiments with indirect calorimetry is described.

The formulae mentioned are not only applicable to the normal metabolism of protein, fat and carbohydrate, but also when other substances, e. g. alcohol, are burned or when pathological final products appear in the urine. For practical use two nomograms are presented.

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From the Laboratory of Zoophysiology, Copenhagen University.

## Microdetermination by Electrolysis of the Sum of Inorganic Cations in Biological Fluids.

By

IB HOLM-JENSEN.

Received 4 March 1943.

The suggestion of determining metals as amalgams formed at a mercury cathode was first made by GIBBS (1880).

SMITH (1903) carried out the first electrolytic determination of alcali metals according to this principle.

HILDEBRAND (1907) designed an apparatus of which the most essential features are given in fig. 1. A glass vessel, the bottom of which is covered with a layer of mercury forming the cathode, is divided into two compartments by a cylindrical glass wall dipping into the mercury, but not reaching the bottom. The solution to be analysed is placed in the central chamber; in

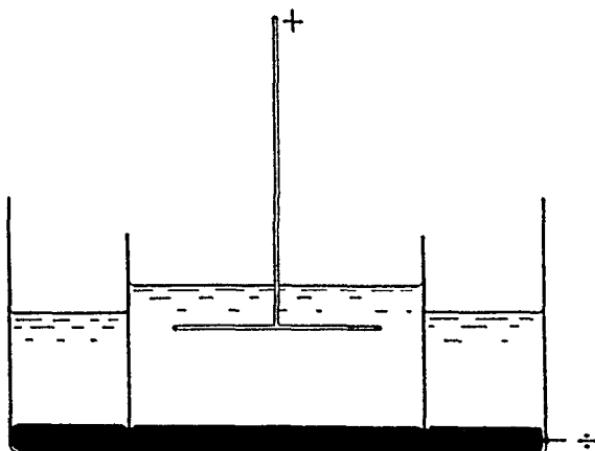


Fig. 1.

the outer compartment may be placed pure water. Certain metals Na, K, Ca, Sr and Ba, which form amalgams of sufficient stability will be redissolved in the outer chamber forming hydroxides, whereas amalgams of Be, Mg and several other metals will be immediately destroyed in the inner chamber,

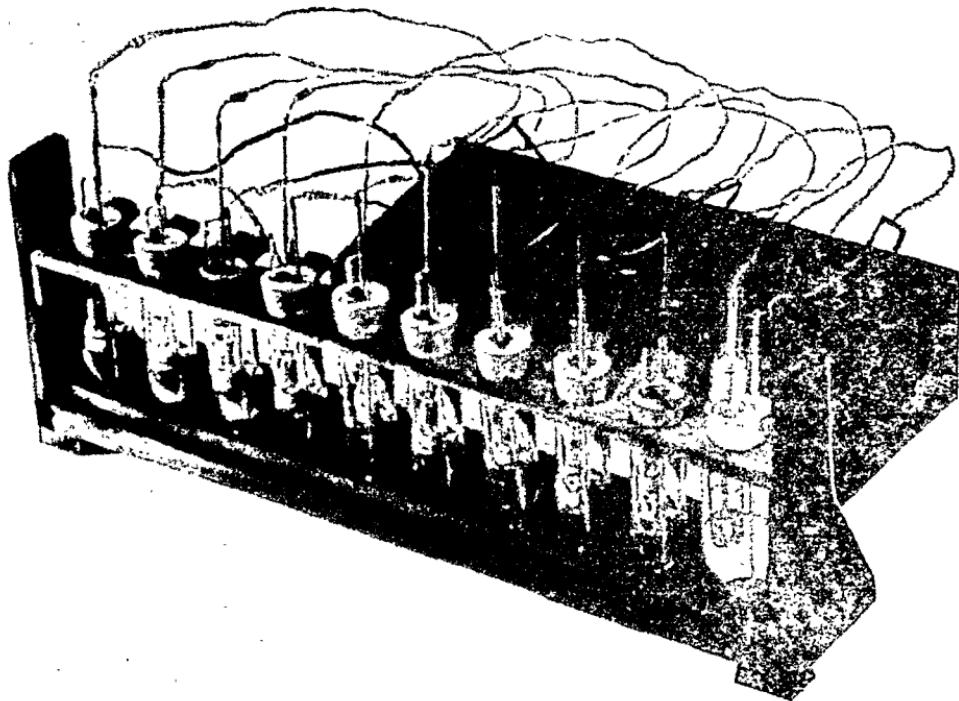


Fig. 2.

and consequently never transmitted to the outer compartment (McCUTCHEON, 1907, LUKENS and SMITH, 1907).

A procedure, for determination of the sum of cations in small samples of biological fluids based on similar principles, was described by ADAIR and KEYS (1934); a more detailed supplement was given by KEYS (1936). In this method, the cations are electrodialysed across a permeable membrane (collodion or cellophane) to a mercury cathode, above which is placed a known amount of standard acid in contact with the mercury. The excess acid is titrated, without removing it from the vessel. Blank determinations must be carried out, they generally amount to less than 0.2 microequivalents. Determinations on samples containing 0.2 ml plasma are accurate within 1 per cent.

A modification of this method was published by NIELSEN (1940). This author claims the same accuracy, but his blanks contained about 2 microequivalents.

I have carried out a number of analyses by the method of ADAIR and KEYS and have been able to reproduce the accuracy stated, using collodion membranes; but using membranes of the kinds of cellophane available, the error increased consid-

erably and the blanks now increased to about 2 microequivalents or even more; they would be diminished only temporarily, when the membranes had previously been used for electrodialyzation, and prolonged washing out, even in acids, proved to be of no effect.

As collodion membranes are rather troublesome to prepare in the way used by ADAIR and KEYS, another method of preparing the membranes was worked out. It was tried also to adapt the method of HILDEBRAND to a micro scale.

In the following an apparatus is described which is designed in accordance with the principles given by ADAIR and KEYS. The apparatus (fig. 2) is adapted for simultaneous determination of 10 samples.

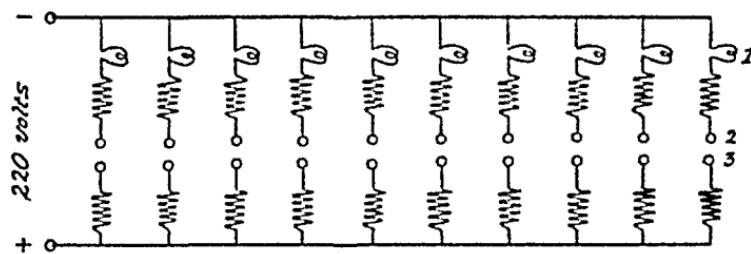


Fig. 3. Diagram of the arrangement of the resistances. 1 is a signal lamp (40 m.amps, 4 volts) which will glow during the first minutes of the electrolyzation and when the electrodes are short-circuited, and thus indicate that the circuit is unbroken. 2 and 3 are connections to the electrodes.

The current used may be either direct current of 110 to 220 volts from the mains, but a high capacity dry battery of at least 110 volts may be substituted. A battery of good quality will last about a year for ordinary use (about 10 analyses a day).

In order to eliminate any danger in touching the electrodes if main current is used and to protect against damage in case of short-circuiting, the current is supplied through suitable resistances e. g.  $2 \cdot 2,500 \Omega$ , which are mounted in a separate box (fig. 3). It is unnecessary to reduce these resistances, when the voltage applied is reduced from 220 to 110 volts as the resistance of the dialyzation unit is about  $10,000 \Omega$  to begin with and is rapidly increasing during the electrolysis. After the lapse of 5 minutes, using 110 volts, it will be of the order of  $50,000 \Omega$ , mainly because the solution is deprived of ions; this means that only a voltage drop of about 10 per cent of the voltage applied, will now take place in the resistance box. From these consid-

erations it is seen also, that it is of practically no use to reduce the resistances during the process.

The dialyzation unit (fig. 4 a) consists of the anode vessel, the cathode vessel, two platinum electrodes sealed into glass tubes and a rubber stopper with perforations for the cathode vessel and for one glass tube carrying the platinum electrode, for the anode.

The anode vessel is a glass tube  $80 \times 25$  mm with excentric tapering.

The cathode vessel is a glass tube  $80 \times 12$  mm open at both ends. One end is constricted by rotating it just touching a gas flame until the inner diameter of the aperture measures  $5.5 \pm 0.5$  mm. (fig. 4 b).

The collodion membrane is prepared directly on the constricted end of this tube from a solution of 10 g collodion in a mixture of 20 ml ether and 80 ml absolute ethyl alcohol. It proved necessary to adhere to this composition especially for the first dipping, and, as evaporation cannot be avoided, the loss should be replaced by a different solution, containing ether and alcohol in a ratio given by their rates of evaporation from the above solution, which means a mixture of 60 ml ether and 40 ml alcohol.

4 dippings are made in the collodion with intervals increasing from 2 to 8 minutes at ordinary room temperature. In the first dipping the tube should only just touch the surface of the solution, whereupon it is immediately suspended in a vertical position with the membrane downwards. The following dippings are successively made deeper up to about 1 cm, and the tubes are rotated about 15—30 seconds in an oblique to horizontal position after each dipping before being again suspended in a vertical position. About 10 minutes after the last dipping a small glass vessel filled with water is placed underneath the tube, and the membrane just made to touch the surface of the water. The simultaneous preparation of 10 of these cathode vessels is finished in about 30 minutes. After the further lapse of 30 minutes they are ready for use. Properly stored, that is with the membranes dipped

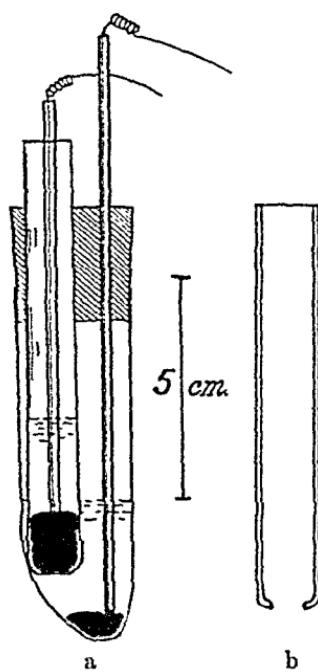


Fig. 4.

about 2 mm into a water bath, the membranes will last for about 10 electrolyses each. If they are allowed to dry the membranes will burst before becoming impermeable.

The electrodes are platinum wires, sealed into glass tubes of  $10 \times 0.25$  cm. The platinum wire is connected through a copper wire with the resistance box.

When assembling the dialyzation unit about 0.4 ml mercury is run into the anode vessel together with 5 ml of pure distilled water, and the sample to be analysed is added. One platinum electrode and the cathode vessel are put through the corresponding perforations of the stopper, about 0.8 ml mercury is placed in the cathode vessel and the stopper is put into position closing the anode vessel. Then exactly 1 ml of a 20 m. n. sulphuric acid is delivered into the cathode vessel. The platinum electrode is introduced resting directly on the collodion membrane and the current can be switched on.

After the lapse of 60 minutes the processes of dialyzation and extraction of the cations from the amalgams into the acid is safely finished with the reservation that it will take 3 hours to obtain 100 per cent of the calcium present.

Titration of the excess acid is now carried out directly in the cathode vessels, which remain in position in the dialyzation units. The platinum electrode should not be removed during the titration. Stirring is obtained by a current of air delivered from a capillary glass tube which dips under the surface of the mercury. Air for bubbling may be obtained by the arrangement shown in fig. 5. A few compressions of the rubber blower will provide sufficient air for bubbling through 10 minutes. If not more than 110 volts has been used for the electrolysis the mercury should be bubbled a few minutes before the titration, otherwise the results will be a little (about 2 per cent) too low. If 220 volts has been applied, this previous bubbling is not necessary. Methyl red is used as indicator. The titration is best made by a REHBERG microburette (1925) containing 100  $\mu$ l about 200—210 m. n. solution of sodium hydroxide; but a burette with a capacity of 1 ml containing 21 m. n. base may be substituted, without seriously diminishing the accuracy of the titration.

Computation of the amount of cations present is carried out in the following way.

The amount of acid placed in the cathode vessel = 20 micro-equivalents. — The volume of base used for titration of excess acid =  $a$ , for titration of blank =  $b$ , and for 20  $\mu$  e. acid =  $c$ .

The content of cations in the sample will be equal to  $\frac{b-a}{c} \cdot 20$  microequivalents. If the volume of the sample is d ml, the concentration of cations will be  $\frac{b-a}{c \cdot d} \cdot 20$  m. n.

If the blanks contain more than 0.2 microequivalents the reason may be that the distilled water contains ammonia. However, this need not impair the precision of the method, as the anode

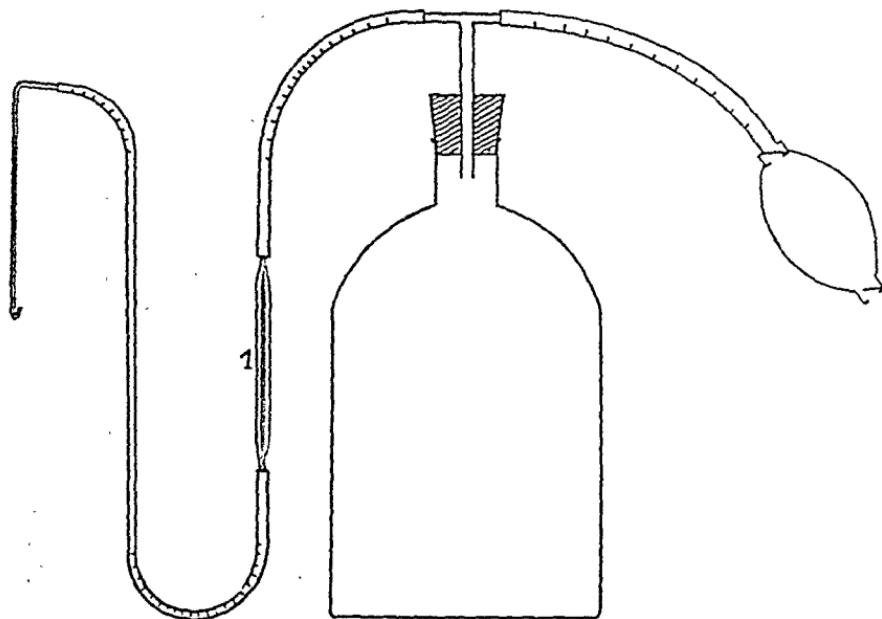


Fig. 5. 1 is a capillary tube for air resistance.

vessels all contain the same quantity of water taken from the same bottle. In most cases it is not necessary to use redistilled water.

Another cause of increased blank determinations may be impurities in the mercury and, as the volume of mercury used cannot conveniently be kept quite constant, the mercury should be cleaned thoroughly. It is first washed with tap water in a beaker then filtered and sprayed twice through a long column of 5 per cent nitric acid and finally through 3 changes of distilled water. This is conveniently done in tubes like that illustrated in fig. 6 (KEYS).

When the mercury had been cleaned in this way, we always got the same blank determination from units containing the usual and 20 times the usual quantity of mercury in the anode vessel;

this means that the mercury does not contribute to the amount determined in the blanks; the blanks were always found to vary in proportion to the volume of water used. Prolonged electrolyzation practically did not influence the blanks.

A large number of determinations on known solutions revealed that  $\text{NH}_4^+$ ,  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Ca}^{++}$  would be determined from pure

solutions with an error rarely exceeding 0.1 microequivalents, whereas only about 20 to 50 per cent of  $\text{Mg}^{++}$ , independent of the presence of other ions, would be transmitted to the acid. The presence of  $\text{Mg}^{++}$  does not seem to disturb the transmission of  $\text{Ca}^{++}$ .

The reason why  $\text{Mg}^{++}$  is partly transmitted in this apparatus, although it is not transmitted in the apparatus of HILDEBRAND is undoubtedly that magnesium-amalgam is decomposed by the fluid in the capillary space between the membrane and the mercury. The magnesium hydroxide formed, being nearly insoluble, will partly adhere to the membrane and partly be carried into the acid. The electroosmotic flow of water through the membrane, about 0.1 to 0.2 ml during the procedure, will support this process.

Analysing 2 or more samples of 0.1 ml of the same blood plasma, the difference will rarely exceed 0.3 microequivalents. Analysing ashed ( $450^\circ$  for 30 minutes) and unashed samples, most of the determinations will give the same agreement. Sodium chloride added to biological fluids is also determined with the same maximum error.

In order to obtain the above accuracy, the delivery of the sample and of the acid must be very precise. For these purposes the syringe pipettes of KROGH (1935) are specially suitable.

A number of experiments were carried out with this electrolyzation apparatus using different designs of cathode vessels. It was tried to substitute the collodion membrane by a glass filter, but the electroosmotic flow of water now exceeded 1 ml/min.

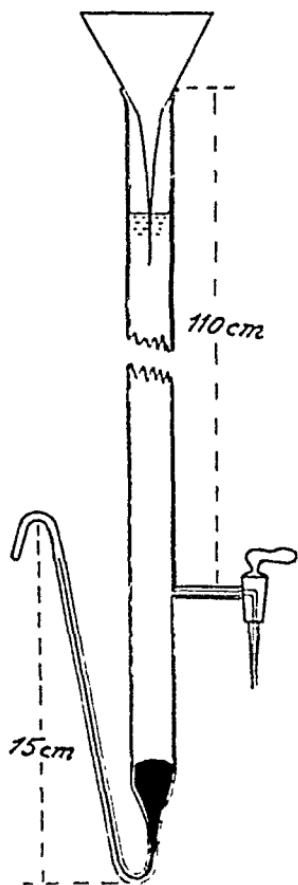


Fig. 6.

The cathode vessel seen in fig. 7 a proved to transmit the ions far too slowly.

The cathode vessel fig. 7 b proved useful for many purposes. The transmission was finished in 1 hour, but the results were too low (about 3 to 6 per cent), this was found to be due to formation of hydroxides of the cations in the film of water present between the glass bottom and the mercury. Gentle stirring during the electrolyzation would diminish this error. A more serious disadvantage analysing blood plasma is formation of airbubbles covering the mercury surface in contact with the fluid in the anode vessel, this often fatally decreased the rate of migration of ions to the mercury. This cathode vessel does not transmit magnesium at all.

I wish to express my hearty thanks to professor AUGUST KROGH, who suggested this study, for his most valuable help and criticism.

### Summary.

A brief survey of the principles of electrolytic determinations of cations of alkali metals and of metals of the alkaline earths is given and a modification of the method of ADAIR and KEYS for microestimation of the sum of cations in unashed samples of biological fluids is presented.<sup>1</sup>

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<sup>1</sup> The Laboratory of Zoophysiology is prepared to supply the apparatus described at a price of 450 Danish kroner inclusive all accessories except battery, burette, mercury and syringe pipettes. A Rehberg microburette costs extra 100 kroner.

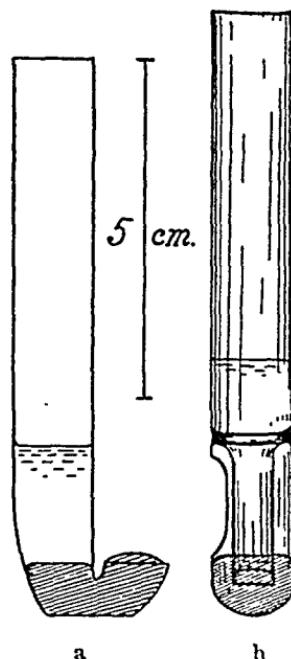


Fig. 7.

From the Central Laboratory of the Sahlgrenska Hospital,  
Gothenburg, Sweden.

## On the Effect of some Coumarin and Dicoumarin Derivatives on the Prothrombin Level in Rabbits.

By

JÖRGEN LEHMANN.

Received 21 March 1943.

In previous papers (LEHMANN 1942 a, b and c), the author has presented data on the prothrombin depressing effect in animals and man of a dicoumarin [3,3'-methylene-bis(4-hydroxycoumarin)], first isolated from moulded sweet clover hay and synthesized by American investigators (CAMPBELL et al. 1940, 1941, STAUFFMANN et al. 1941) who also first produced hypoprothrombinemia in animals with this substance. The author introduced the dicoumarin in the therapy of thrombosis (l. c.) and presented 17 cases, in most of which a definite beneficial effect was demonstrated. At present more than 100 cases of thrombosis have been treated and in post-operative prophylactic purpose about 200 patients. Also American investigators seem to be interested in the therapeutic use of the substance. A personal communication from dr. ALLEN and dr. BARKER at the Mayo clinic, Rochester, mentions, that they have treated a similar amount of patients.

Parallell to the clinical work the author has run a study intending 1) to investigate derivatives of the simple coumarin molecule in order to see if any antiprothrombin action could be demonstrated, 2) to find the active groups in the 3,3'-methylene-bis(4-hydroxycoumarin), 3) to investigate the possibilities of finding a watersoluble stable derivative of the dicoumarin for intravenous injection and eventually to find other active dicoumarin compounds. The present paper deals with these problems.

### Methods and Preparations.

Rabbits on ordinary diet were used as test animals and the prothrombin was determined with the authors' modification (LEHMANN 1941) of QUICK's method. The prothrombin level was expressed in *index* units.

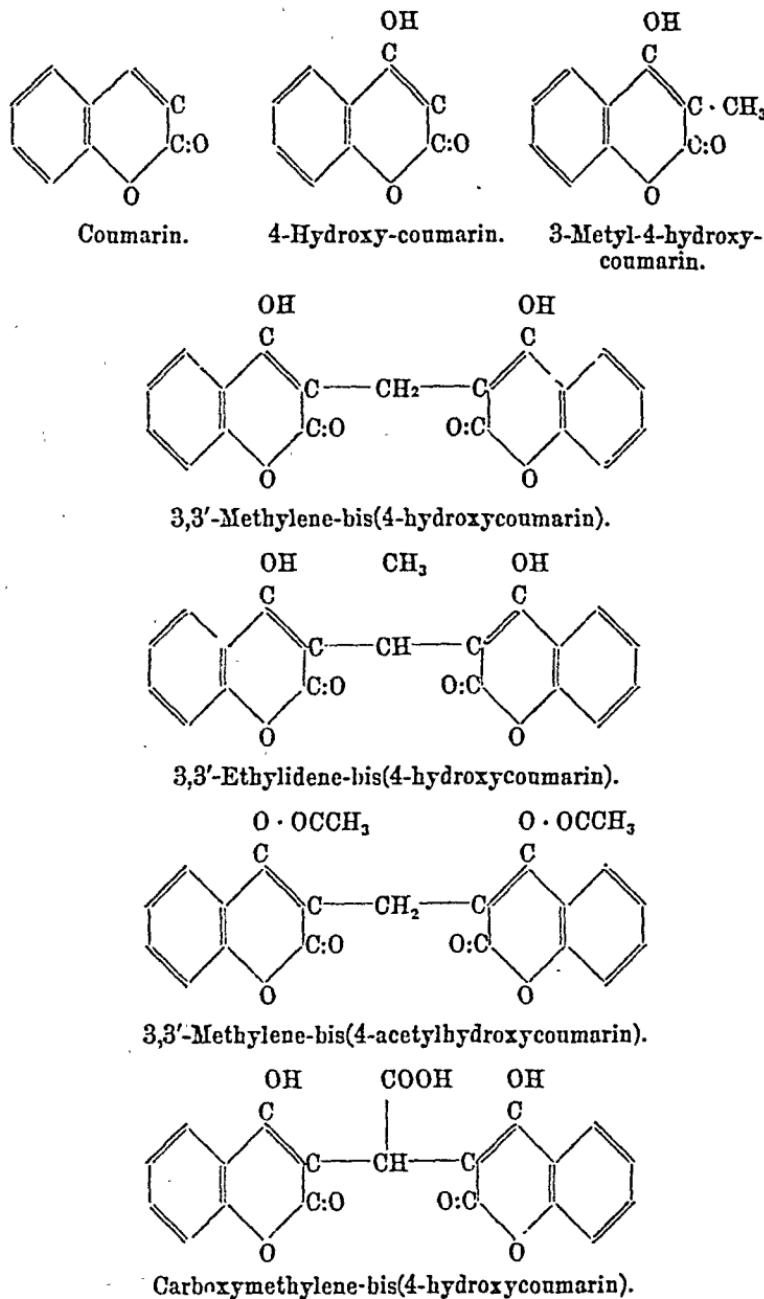


Fig. 1.

The substance to be tested was given per os in increasing doses to the same rabbit. After every dose the prothrombin was allowed to return to normal index (90—110) and after 2—4 days a new dose was given.

In order to be able to compare the activity of the different substances a unit for their antiprothrombin action was chosen. This unit is defined as the amount of the substance, expressed in mg, and given per os to rabbits of 2 kg body weight, which depresses the index from 100 to 50 ( $\pm$  10). Abbreviated the *antiprothrombin unit* is termed AP.U. Such a unit is open for criticisms from several points of view (different sensibility of the animals, the hyperbolic index scale etc.). The unit therefore only serves as a preliminary, very rough basis for the comparison.

The substances tested were — with exception of coumarin — kindly synthetized by K. G. ROSDAHL and T. NILSSON at the *Ferrosan Comp.*, Malmö, Sweden:

- 1) *Coumarin*, recrystallized from water. M. p. 70°C.
- 2) *4-Hydroxycoumarin*. Prepared by the method of PAULY and LOCKEMANN (1915) from the methyl ester of acetylsalicylic acid and metallic sodium. Recrystallized from water. M. p. 210°C.
- 3) *3-Methyl-4-hydroxycoumarin*. Prepared by the method of HEILBRON and HILL (1927) from ethylmethylsodioacetate and o-acetoxybenzoylchloride. Repeated recrystallization from diluted ethanol and finally from methanol. M. p. 229°C.
- 4) *3,3'-Methylene-bis-(4-hydroxycoumarin)*. Prepared by the method of ANSCHÜTZ (1909) from 4-hydroxycoumarin and formaldehyde. M. p. 285°C.
- 5) *3,3'-Ethylidene-bis(4-hydroxycoumarin)*. Prepared by the method of ANSCHÜTZ from 4-hydroxycoumarin and acetaldehyde. Recrystallized from aceton. M. p. 176—177°C.
- 6) *3,3'-Methylene-bis-(4-acetylhydroxycoumarin)* and
- 7) *Carboxymethylene-bis-(4-hydroxycoumarin)* were prepared by K.-G. ROSDAHL by methods to be published later.

## Results.

1) *Coumarin*. Fig. 2 shows the action of coumarin on the prothrombin level. As seen 1 AP.U. = 2000 mg. The result was rather irregular in different rabbits. Some of the rabbits died from this dose and most of them when it was increased to 2500 mg.

### 2) *4-Hydroxycoumarin*.

The introduction of an hydroxy group in position 4 increased the antiprothrombin activity of the coumarin about 4 times. 1 AP.U. = ca. 500 mg. See fig. 3.

### 3) *3-Methyl-4-hydroxycoumarin*.

This compound is of special interest as its constitution is very similar to that of the vitamin K (3-methyl-1,4-naphthoquinone).

According to this similarity in constitution it could be expected, that the substance should be able to interfere with vitamin K in the hypothetic enzyme complex of the liver, in which vitamin

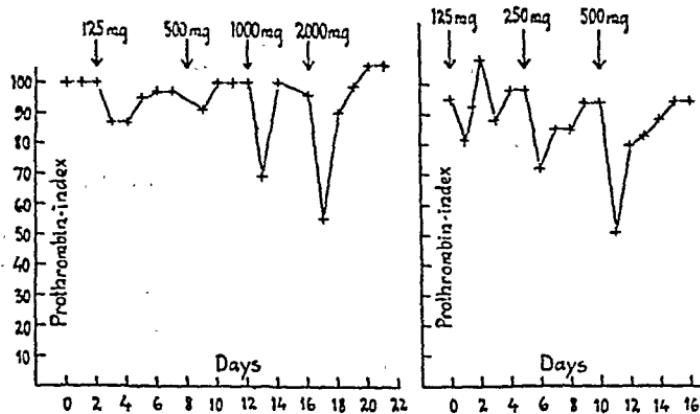


Fig. 2. The effect of coumarin on the prothrombin level in rabbit.

Fig. 3. The effect of 4-hydroxycoumarin on the prothrombin level in rabbit.

K is active in producing prothrombin (Competitive inhibition, see further LEHMANN 1942 a, b and c).

Fig. 4 shows that these suggestions were not verified as the substance does not depress the prothrombin level more than 4-hydroxycoumarin. 1 AP.U. = ca. 500 mg.

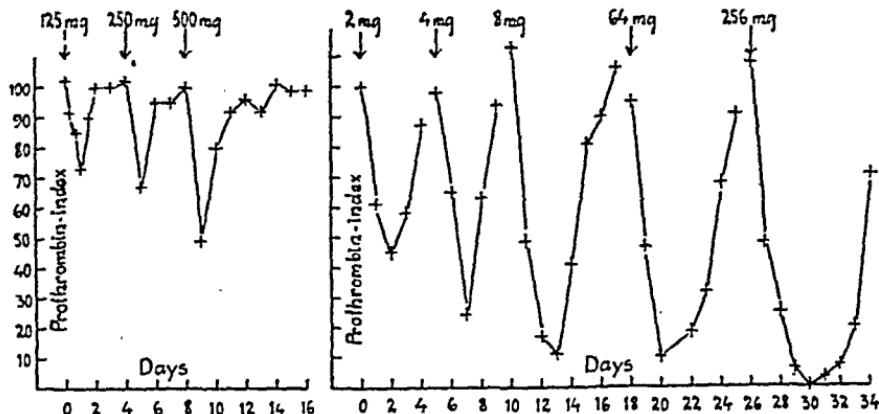


Fig. 4. The effect of 3-methyl-4-hydroxycoumarin on the prothrombin level in rabbit.

Fig. 5. The effect of 3,3'-methylene-bis-(4-hydroxycoumarin) on the prothrombin level in rabbit.

The intervals in the prothrombin curve between the different doses indicates that a few days passed between the doses.

Further substitution in the coumarin molecule was without interest.

4) *3,3'-Methylene-bis-(4-hydroxycoumarin)*.

In fig. 5 is seen a typical experiment with this substance.

1 AP.U. = 2—4 mg. Lethal dose about 500 mg. The author has not found any rabbit resistant to the substance, as mentioned by the American investigators.

5) *3,3'-Ethylidene-bis-(4-hydroxycoumarin)*.

The substitution of the methylene bridge by an ethylene bridge between the two coumarin molecules gave a substance of about

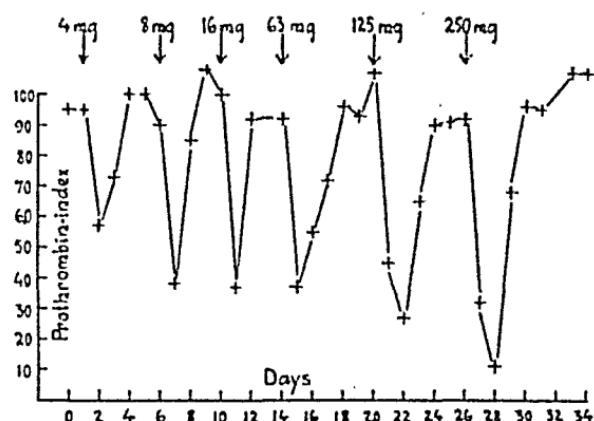


Fig. 6. The effect of 3,3'-ethylidene-bis-(4-hydroxycoumarin) on the prothrombin level in rabbit.

the same activity as that of the substance with the methylene bridge. The shape of the prothrombin curve, the toxicity and the solubility was similar to that of the original dicoumarin. Fig. 6 shows the activity of the substance.

6) *3,3'-Methylene-bis-(4-acetylhydroxycoumarin)*.

Substitution in the two 4-hydroxygroups was tried with several acids. Phosphorylation and sulphonation was, in analogy with the synthetic vitamin K, expected to give watersoluble substances.

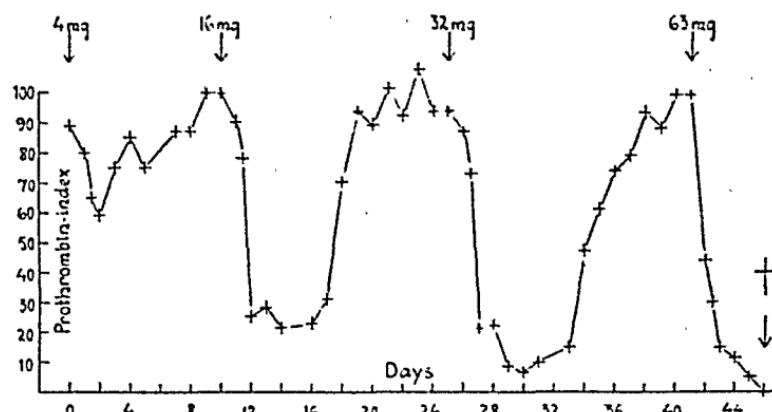


Fig. 7. The effect of 3,3'-methylene-bis-(4-acetylhydroxycoumarin) on the prothrombin level in rabbit.

These substitutions have, however, as yet not been successfully performed. Acetylation was easily performed, but the substance was not watersoluble. The activity of this compound is seen in fig. 7.

1 AP.U. = ca. 4 mg. thus about the same activity as for the unsubstituted 3,3'-methylene-bis-(4-hydroxycoumarin). It differs however from this substance in two respects. a) The duration of the produced hypoprothrombinemia persists considerably longer (4—7 days) as compared with 1—2 days for the 3,3'-methylene-bis-(4-hydroxycoumarin) before it suddenly rises towards normal index-values. b) The toxicity was found increased as the dosis lethalis was about 50 mg, compared with about 500 mg for the 3,3'-methylene-bis-(4-hydroxycoumarin) — thus 10 times more toxic. Severe lesions were found in the liver and the kidneys.

These properties prohibit its use in the clinic.

### 7) Carboxy-methylene-bis-(4-hydroxycoumarin).

The introduction of a carboxy group in the methylene bridge gave a substance, whose sodium salt was watersoluble to 25 %. The substance was however of very little activity by intravenous injection. A somewhat greater activity was found when administered per os. (Fig. 8.) 1 AP.U. = 150—300 mg.

The substance was, due to its low activity, of no interest.

## Discussion.

The dimethyl ether of the dicoumarin has been investigated by CAMPBELL and LINK (1941) and found inactive. The authors therefore conclude that the hydroxygroups are essential for the prothrombin depressing effect. However, acetylation in the hydroxygroups was in this investigation found to give a highly active substance with a prothrombin curve differing from that of the not substituted dicoumarin. See fig. 7. It can be discussed if the prolonged prothrombin depressing effect is due to a successive hydrolysis of the acetylated compound giving the 3,3'-methy-

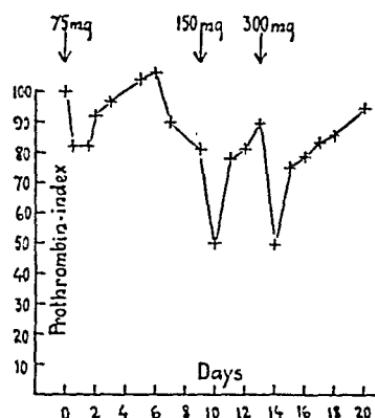


Fig. 8. The effect of carboxymethylene-bis-(4-hydroxycoumarin) on the prothrombin level in rabbit.

lene-bis-(4-hydroxycoumarin). This process can not explain the fact, that the acetylated compound is about 10 times more toxic than the not acetylated as judged from the lethal dose. On the other hand, it is just as possible, that the not substituted compound to a certain degree is acetylated in the organism. Any sure conclusion concerning the necessity of the hydroxygroups for the antiprothrombin activity thus can not be drawn from these experiments.

Since the present investigation was finished JANSEN and JENSEN (1942) have published a similar investigation. The substances Nr. 2—3—4 and 5 were also tested by these authors. Only 3,3'-methylene-bis-(4-hydroxycoumarin) and the ethylidene compound were found active. That JANSEN and JENSEN did not find 4-hydroxycoumarin and 3-methyl-4-hydroxycoumarin active is explained by the circumstance, that they have only tested doses of 10 centigrams per kilo body weight (rabbits) and thus have not been able to detect the slight activity of these compounds demonstrated by higher doses.

JANSEN and JENSEN discusses the present author's theory of the mechanism of the prothrombin depressing effect of the dicoumarin. According to this theory the dicoumarin — owing to its structural relation to vitamin K — acts in a competitive way preventing the interaction between the vitamin and the enzyme system responsible for the prothrombinproduction in the liver. On the basis of certain differences in the chemical structure of the two substances the Danish authors do not accept the theory. Especially the inactivity of 3-methyl-4-hydroxycoumarin is taken as an argument against the theory. As shown here this substance has a certain activity even if, from its structural relation to vitamin K, it would be expected to be more active than found. When discussing the theory it must be remembered, that even if the chemical structure of a substance often can explain its pharmacological activity, it is difficult to deduce an inhibitory effect of a closely related substance from the chemical constitution. The group or groups to be changed to produce the inhibitory effect are not known in this case. The best way to judge the validity of the theory must therefore be to test, if the two substances counteract one another. As shown by the author this is the case with the dicoumarin and vitamin K in man.

The introduction of a *methyl* group in the methylene bridge did not change the activity essentially. However, if a *carboxy* group

was introduced — giving a watersoluble substance — the activity decreased remarkably. If this is due to a splitting of the two coumarin molecules in the organism is not known. That the substance was found more active given per os than parenterally is explained by the fact, that all of the substance passes the liver when given per os.

The very slight activity of the simple coumarin molecule is perhaps not specific. Possibly other substances given in so high doses will be able to produce a similar disturbance in the prothrombin production in the liver.

As seen from the experiments the compounds investigated showed no advantages compared with the original 3,3'-methylene-bis-(4-hydroxycoumarin) and an active stable watersoluble substance for intravenous injection was not found.

### Summary.

1) The prothrombin depressing effect of derivatives of coumarin and 3,3'-methylene-bis-(4-hydroxycoumarin) have been investigated in rabbits in order to find the pharmacologically active groups and if possible a watersoluble stable compound of the dicoumarin.

2) Most of the substances tested were found active. The highest activity — combined with less toxicity — was exerted by 3,3'-methylene-bis-(4-hydroxycoumarin).

3) Carboxymethylene-bis-(4-hydroxycoumarin) was watersoluble but of very little activity.

4) The active groups in the dicoumarin are discussed. Introduction of certain radicales in the hydroxygroups as well as in the methylene bridge did not abolish the activity whereas this was the case when other radicales were introduced at the same places.

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## Oxidative Breakdown of Hyaluronic and Chondroitin Sulphuric Acid.

By

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Received 25 March 1943.

The term "spreading factor" has in later years been applied to substances with the power of increasing the spreading of dye-stuffs or toxins in the skin when injected intracutaneously together with these. Such a substance was first found in normal testicular tissue by DURAN-REYNALS (1928) and has later been demonstrated in various other tissues, as well as in certain bacteria and in snake venoms. Moreover, substances such as ascorbic acid (oxidised by hydrogen peroxide), azoproteins, diazobenzene-sulphonic acid and arsenious oxide have been found active as spreading factors (MCLEAN and HALE, 1940; MCLEAN and HALE 1941; FAVILLI, 1940; HOBBY, DAWSON, MEYER and CHAFFEE, 1941).

CHAIN and DUTHIE (1940) made the important observation that testicular extracts possessing spreading activity decrease the viscosity of synovial fluid and vitreous humour with the liberation of reducing substances. MEYER and CHAFFEE (1940) later showed that the hyaluronic acid present in these substrates is split into reducing products by such extracts, and it has subsequently been demonstrated that extracts of leech head, skin and pneumococci, all containing spreading factors, also hydrolyse hyaluronic acid (HOBBY, DAWSON, MEYER and CHAFFEE, 1941; MEYER, CHAFFEE, HOBBY and DAWSON, 1941). As this polysaccharide acid is present in the skin (MEYER and CHAFFEE, 1941) the spreading effect in all these instances is not improbably due to a hyaluronidase action.

Ascorbic acid, azoproteins, and diazobenzenesulphonic acid have also been shown to decrease the viscosity of synovial fluid, which suggests that these substances might also exert their spreading action by some form of depolymerisation of the hyaluronic acid. In these cases, however, the "mucinase" action cannot be due to a simple hydrolysis of the hyaluronic acid, since according to McCLEAN and HALE (1940) the decrease of viscosity of the substrates is not accompanied by the liberation of free N-acetylglycosamine or other reducing substances. Although the action of ascorbic acid on synovial fluid and vitreous body mucin has been studied in some detail, the mechanism of the reaction is still obscure. There has not even been any definite experimental proof that the decrease of viscosity is due to a breakdown of the hyaluronic acid.

ROBERTSON, ROPES and BAUER (1939) found that the viscosity of vitreous humour was reduced spontaneously and suggested that this reduction was due to its content of ascorbic acid (approximately 0.5 mg/ml as determined by dichlorophenolindophenol titration). They therefore studied the effect of ascorbic acid on the viscosity of mucins from vitreous body and synovial fluid. They found that the viscosity reducing effect of ascorbic acid was activated by a trace of copper or hydrogen peroxide. An excess of the latter decreased the activity. Potassium ferricyanide, iodine or quinone could not serve as activators. Ascorbic acid also decreased the viscosity of mucins of epithelial origin as well as that of starch solutions. Increasing the concentration of the ascorbic acid from M/500 to M/5, McCLEAN and HALE (1940) found a quite inconsiderable rise in the reaction rate. The viscosity reached at the end of the experiments did not rise again on dialysis of the reaction mixture. A reversibility of the reaction could therefore not be demonstrated in this way. A series of other reducing substances, hydrogen sulphide, hydroquinone, pyrogallol and sodium sulphite were also found to be more or less active in the same way as ascorbic acid. Just as this compound they did not liberate any reducing substances from the substrate. Even the viscosity of the heated substrates was reduced, which excluded the possibility that an enzyme already present was activated by these agents.

It appeared to us that the decrease of viscosity brought about by ascorbic acid in the substrates concerned might be due to an oxidative breakdown of the hyaluronic acid. Such a reaction

would not be unparalleled, since cellulose is known to be easily depolymerised by various oxidising agents. An oxidative breakdown of hyaluronic acid might also be the cause of the spreading effect of other substances without hydrolysing power. As the reaction between ascorbic acid and hyaluronic acid might also be of biological importance, it seemed well worth a closer study. It may be stated here that the idea suggested above concerning the action of ascorbic acid proved to be correct, and that a similar breakdown of the hyaluronic acid could be effected by various oxidising agents, i. a. by molecular oxygen. In view of the close chemical and biological kinship between hyaluronic and chondroitin sulphuric acids, the latter was also included in our investigations.

We had already finished our experimental work, when we observed a further publication by ROBERTSON, ROPES and BAUER (1941) dealing with ascorbic acid as a degrader of mucins. Ascorbic acid together with hydrogen peroxide gave a rapid degradation, whereas hydrogen peroxide alone was found to be inactive. Ascorbic acid oxidised by atmospheric oxygen was also inactive. (This oxidation, however, had proceeded too far to yield reversible oxidation products.) The degradation caused by ascorbic acid without hydrogen peroxide was found to be completely inhibited by catalase. Hence the authors conclude that the ascorbic acid causes degradation only in its reduced form and in the presence of hydrogen peroxide. The mechanism could not be explained. Pointing to the resemblance between the degradation of mucin and the reduction of the viscosity of starch paste by amylophosphatase and between the breakdown of synovial fluid mucin by serum phosphatase and the dephosphorylating activity of ascorbic acid, they suggest that a dephosphorylation might be responsible for the breakdown of the mucins. As will be seen from the following data, the observations of ROBERTSON, ROPES and BAUER (1941) are in several respects not in agreement with ours.

### Methods.

As substrates we used pure hyaluronic acid isolated from vitreous body, synovial fluid and umbilical cord and chondroitin sulphuric acid from cattle cartilage.<sup>1</sup>

<sup>1</sup> We are indebted to Professor G. BLIX for supplying this material. The purity was checked by analysis (BLIX). The substances were isolated without the use of alkali. For the methods of preparation we refer to BLIX and SNELLMAN (1943).

Viscosities were determined with Ostwald viscosimeters in a water bath usually at  $37 \pm 0.1^\circ$  C. All viscosities were calculated relative to the viscosity of the buffer employed (M/15 phosphate buffer of SØRENSEN). As a measure of the viscosity reducing activity was employed the time required for the liquid to arrive at a level half-way between the original level and that of the solvent.

Total reduction calculated as glucose was determined by the method of HAGEDORN and JENSEN. Acetylglucosamine was determined by the method of MORGAN and ELSON (1934). Glucosamine was determined according to NILSSON's modification of this method (1936), glucuronic acid according to BURKHART, BAUR and LINK (1934).

A few drops of toluene were always added in the experiments to prevent bacterial action.

### Experiments on Hyaluronic Acid.

1. *The effect on the viscosity of hyaluronic acid by ascorbic acid, hydrogen peroxide and molecular oxygen.* — The results of our first series of experiments are given in Fig. 1. It appears that ascorbic acid decreases the viscosity of solutions of pure hyaluronic acid, and that the effect is highly accelerated by small amounts of hydrogen peroxide. With ascorbic acid alone the half viscosity level was reached in 55 minutes, with ascorbic acid + hydrogen peroxide in less than 5 minutes. Contrary to the findings of ROBERTSON et al. for mucin, hydrogen peroxide alone is here seen to cause a marked fall in the viscosity, although this effect is relatively slow, the half viscosity level being reached in about 150 minutes.<sup>1</sup> As in the case of the mucin, traces of Cu<sup>++</sup> accelerated the effect of ascorbic acid. When ascorbic acid was used without hydrogen peroxide, the effect was completely inhibited on addition of small amounts of diethyldithiocarbamate or potassium cyanide. The activity could in these cases be restored by an excess of copper. On the other hand tungstate, which forms complexes with iron but not with copper (BANGA, 1938), did not have any inhibitory effect. These observations indicate that the decrease of viscosity in the experiments where only ascorbic acid was used, was very probably due to a copper catalysis.

In agreement with the results of McCLEAN and HALE (1941) and MADINAVEITIA and QUIBELL (1941) for mucins, we found

<sup>1</sup> The time required to reach half viscosity level is somewhat variable for different preparations of hyaluronic acid.

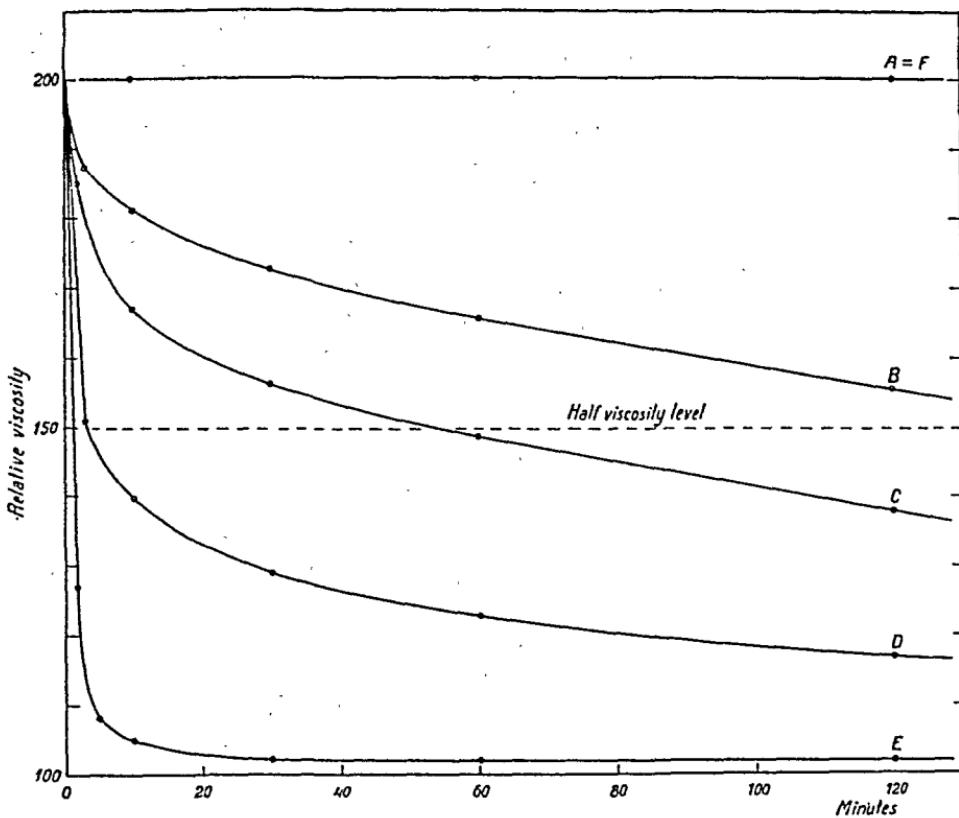


Fig. 1.

Hyaluronic acid 0.25 % in all experiments. A = control, buffer only, pH 7.2. B = 0.1 ml 3 %  $H_2O_2$  in 5 ml solution, (no ascorbic acid). C = M/100 ascorbic acid. D = M/100 ascorbic acid plus traces of  $Cu^{++}$ . E = M/100 ascorbic acid +  $H_2O_2$ , in the same conc. as in B. F = M/100 ascorbic acid + a small amount of diethyldithiocarbamate or potassium cyanide.

the time required to bring about a given decrease of viscosity of a hyaluronic acid solution was but little influenced by variation within a wide range of the ascorbic acid concentration. Thus no noteworthy increase in the reaction rate occurred when the concentration of the ascorbic acid was raised from M/3,000 to M/50. With M/5,000 or M/10,000 ascorbic acid the reaction rate showed, however, a marked fall. The most probable explanation for these facts seems to be that the concentration of  $Cu^{++}$  is a limiting factor in the experiments where the ascorbic acid concentration exceeds M/3,000.

Further experiments showed that the presence of molecular oxygen is necessary to produce the ascorbic acid effect when hydrogen peroxide is not added.

As seen from Fig. 2 no diminution of viscosity occurred under anaerobic conditions. The bubbling of oxygen through the liquid increased the reaction rate somewhat.

The results obtained above suggested that the dehydroascorbic acid might be the oxidising agent. This question was further investigated as follows. Molecular oxygen was bubbled through a M/200 solution of ascorbic acid in phosphate buffer of pH 7.2 at + 37° C. The bubbling was stopped when a sample taken from the reaction mixture showed by dichlorophenolindophenol titra-

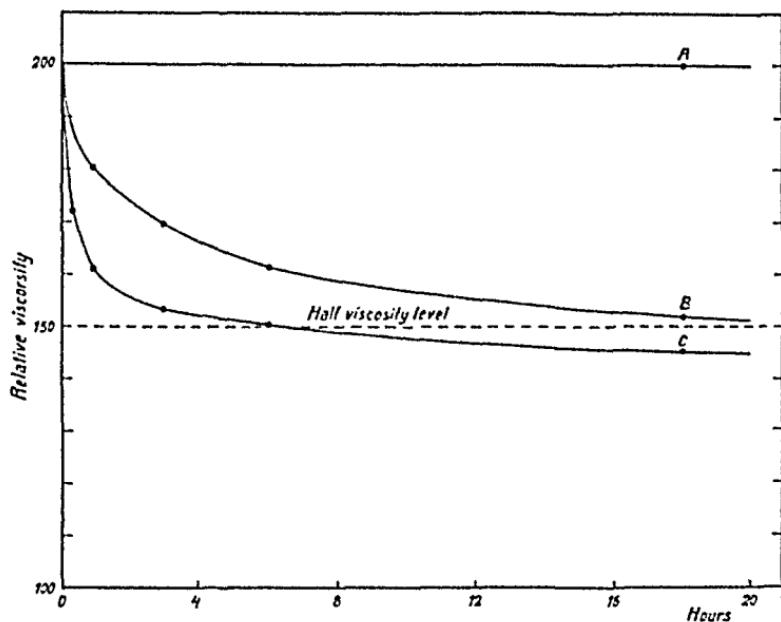


Fig. 2.

Hyaluronic acid 0.25 % in buffer of pH 7.2. Temp. + 37° C. Ascorbic acid M/100 in all experiments. No extra Cu<sup>++</sup> was added. A = in vacuo. B = open to air. C = oxygen bubbled through the mixture.

tion that at least 99 % of the ascorbic acid originally present had been oxidised. The viscosity reducing power of this oxidised solution proved to be practically the same as that of the original one (Fig. 3). To exclude the possibility that the remaining un-oxidised ascorbic acid (at most M/20,000) was responsible for the reaction, a control experiment with M/10,000 ascorbic acid was carried out. This concentration, however, was practically inactive. — From Fig. 3, where these experiments are graphically reproduced, it also appears that the ascorbic acid fully oxidised by oxygen is, at least in the concentrations here used, not far from inactive in the absence of molecular oxygen.

The small and somewhat varying effect obtained in these anaerobic experiments might be due to some oxidation of hyaluronic acid by dehydroascorbic acid. In view, however, of the relatively high concentration of copper here used and the fact that the viscosimetric determinations were not performed with exclusion of air, such an explanation of the small effect observed does not appear very probable. In fact we did not succeed in demonstrating by titration any reappearance of ascorbic acid in these experiments. In the aerobic experiments with oxidised ascorbic acid no reappearance of ascorbic acid could be established by titration. Thus we have obtained no evidence as yet that dehydroascorbic acid functions as oxidiser in the reaction studied. It may be added that the oxidation of the ascorbic acid by molecular oxygen, as determined by dichlorophenolindophenol titration, did not proceed more slowly in the presence of hyaluronic acid than in its absence. This observation also refutes

to some degree the idea that the dehydroascorbic acid functions here as an oxidising agent, and that ascorbic acid — dehydroascorbic acid serves as a "cyclic" catalyst. The fact that the oxidised ascorbic acid was active only under aerobic conditions might possibly indicate that a labile peroxide is involved in the reaction. The observation that the activity of oxidised ascorbic acid is almost completely inhibited by diethyldithiocarbamate indicates that the copper does not confine its activity to that of an ascorbic acid oxidase.

The observation that ascorbic acid, to which had been added an equivalent amount of iodine or potassium ferricyanide, was

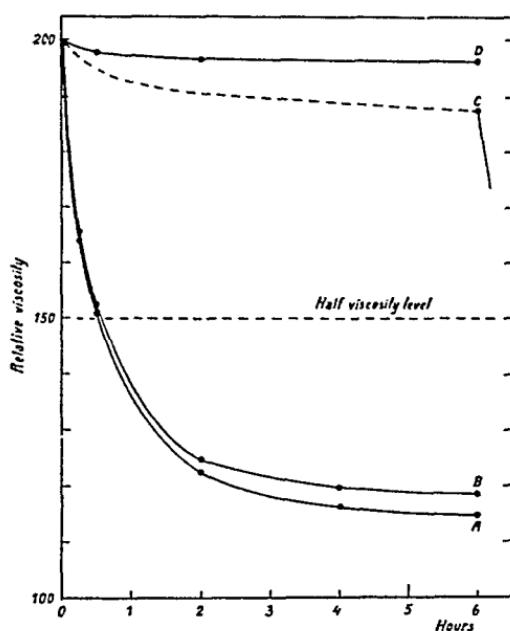


Fig. 3.

Hyaluronic acid 0.25 %. A = M/200 ascorbic acid + traces of  $\text{Cu}^{++}$ . Free access of atmospheric oxygen. B = M/200 ascorbic acid oxidised by molecular oxygen. C = as B but in vacuum. D = control experiment with M/10,000 ascorbic acid.

inactive, is of some interest in this connection. As hydrogen peroxide is formed by autoxidation of ascorbic acid (BARRON, DE MEIO and KLEMPERER, 1935) it appeared conceivable that hydrogen peroxide might also play a rôle in the experiments where it had not been added. This possibility could, however, be ruled out, since the addition of pure catalase<sup>1</sup> did not inhibit the action of ascorbic acid in such experiments. In this respect our results disagree with those of ROBERTSON, ROPES and BAUER for mucin (1941).

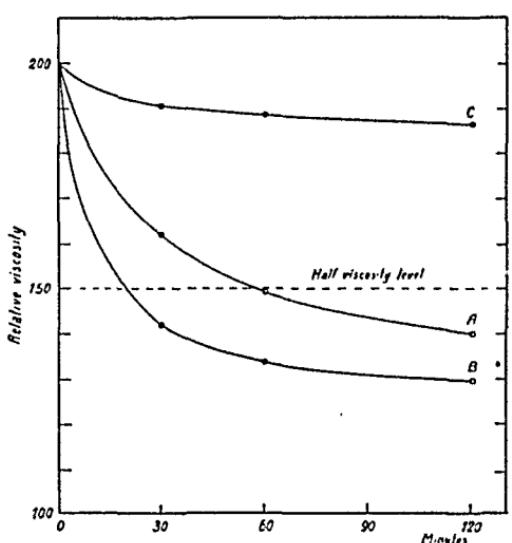


Fig. 4.

Hyaluronic acid 0.3 %. pH 7.2. A = buffer only. B = A plus traces of copper. C = A plus 1 mg Na-cyanide or 1 mg diethyldithiocarbamate in 5 ml. solution.

A solution of hyaluronic acid in a phosphate buffer of pH 7.2 standing open to the air did not change in viscosity within the observation time of one week. Bubbling oxygen through the liquid at + 37° C for 24 hrs. produced a small (about 5 %) reduction of viscosity. At + 100° C the effect was marked, the half viscosity being reached in 1—2 hrs. The experiments were repeated in buffers with the pH varying between 5 and 8. The decrease in viscosity was somewhat more rapid in the more alkaline solutions than in the acid ones. Control experiments with nitrogen instead of oxygen gave an inconsiderable reduction in viscosity. In order to find out whether a copper catalysis was also involved in the oxidation by molecular oxygen, experiments were conducted at + 100°

A series of experiments were conducted in which hydrogen peroxide was tried alone in various concentrations. With M/5 H<sub>2</sub>O<sub>2</sub>, the half viscosity value was reached after 75 min.

A decrease of viscosity was observed already at a concentration of hydrogen peroxide as low as M/500. In these experiments the reaction rate was also raised by traces of Cu<sup>++</sup> and strongly inhibited by cyanide and diethyldithiocarbamate.

<sup>1</sup> We are indebted to Professor H. THEORELL, Stockholm, for the pure catalase preparation used.

C in which minute amounts of diethyldithiocarbamate and cyanide respectively were added. The results given in Fig. 4 indicate that the autoxidation of hyaluronic acid at + 100° C is, at least in the main, due to a heavy metal catalysis.

*2. The effect of certain other oxidising agents on the viscosity of hyaluronic acid:*

— Some redox systems of the same type as ascorbic acid — dehydroascorbic acid were also tried. Cysteine in the presence of copper had at pH 7.2 a marked effect. Hydroquinone and pyrogallol at pH 7.2 had an obvious but not very strong viscosity reducing effect, which like that of ascorbic acid disappeared under anaerobic conditions. We also observed that the solution with hyaluronic acid and hydroquinone kept colourless for a much longer time than did a blank solution with hydroquinone and buffer only. The explanation for this is probably that the quinone formed in the oxidation of hydroquinone by atmospheric oxygen is reduced by the hyaluronic acid, hydroquinone-quinone thus acting as a cyclic catalyst. Some other substances of a related chemical type, namely adrenaline, dioxyphenylalanine and thioglycollic acid were, on the other hand, found to be inactive (at pH 7.2). An experiment with a stronger oxidising agent, potassium permanganate, brought about a marked decrease of viscosity. Some of these results are given in Fig. 5.

*3. The degradation products of hyaluronic acid.* — In the experiments with ascorbic acid, hydrogen peroxide, and molecular oxygen the reaction mixtures were tested at the end of the experiment for reducing substances and free acetylglucosamine, in all instances with negative results. That depolymerisation of

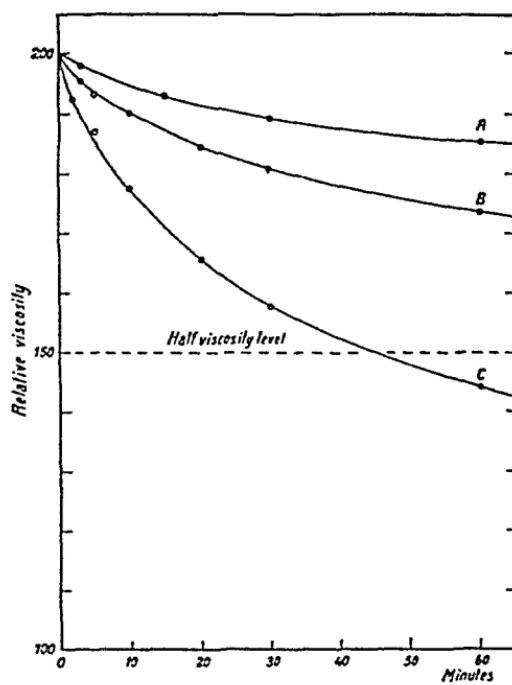


Fig. 5.

Hyaluronic acid 0.3 %. pH 7.2. A = M/50 hydroquinone plus traces of Cu. B = M/150 cysteine hydrochloride plus traces of Cu. C = M/100 potassium permanganate.

the hyaluronic acid takes place as the viscosity of the solutions fall is, however, easily demonstrated.

The Na-salt of hyaluronic acid was dissolved in distilled water and dialysed against running tap water for 48 hours. The nitrogen content of the solution determined by the Kjeldahl method remained constant during this period. 100 ml of the solution, containing 80 mg hyaluronic acid, were treated with ascorbic acid at 37° for 24 hours and thereafter dialysed against distilled water. N-containing substances passed into the external water, and after dialysis for 24 hours against running water the N-

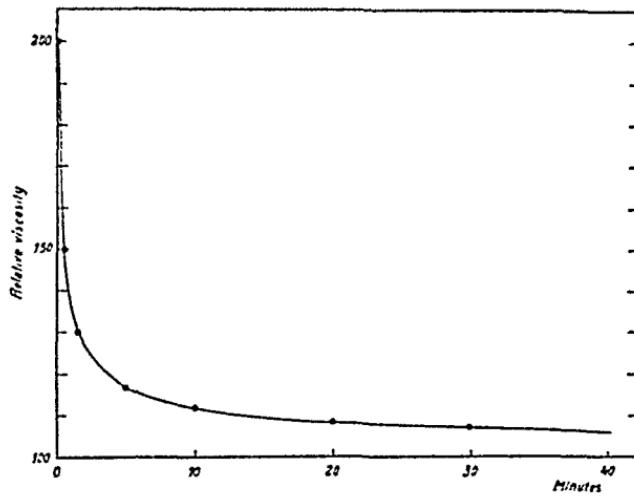


Fig. 6.

content of the internal solution had fallen to 24 % of the original value. When ascorbic acid + hydrogen peroxide was used still more N-containing substances passed through the dialysis membrane.

A more direct proof of the depolymerising action of the ascorbic acid was obtained in the following way. As has been shown by BLIX and SNELLMAN (1943), solutions of hyaluronic acid show a very marked double refraction of flow, indicating molecules of a threadlike shape. In the experiment given in Fig. 6 the reaction mixture contained 1 % hyaluronic acid as Na-salt, 0.5 % ascorbic acid and an equimolar amount of  $H_2O_2$  in a phosphate buffer of pH 7.2. The temperature was + 37° C. The change of double refraction was followed simultaneously in the same solution, and was found to diminish rapidly and to be almost unmeasurable after 20 minutes. Similar results were obtained using ascorbic acid alone or  $H_2O_2$  alone. Here also there was

good agreement between the fall in viscosity and the decrease and disappearance of double refraction.<sup>1</sup>

The formation of carboxyl groups is to be expected in an oxidative breakdown of a polymeric carbohydrate. Accordingly experiments were conducted to find out whether the formation of such groups could be detected by titration in the degradation of hyaluronic acid by ascorbic acid or hydrogen peroxide.

50 mg hyaluronic acid in 10 ml water were exactly neutralised with N/100 NaOH, using bromothymol blue as indicator. 0.15 ml 30 % hydrogen peroxide was added and the mixture kept at + 37° C, protected against atmospheric carbon dioxide. At appropriate intervals the reaction mixture was titrated with N/100 NaOH. The degradation was at the same time followed viscosimetrically. The results are given in Table I.

Table I.

Time	ml N/100 NaOH required for neutralisation
24 hrs.	4.8
48 "	4.1
72 "	2.5
96 "	0.7
	Total 12.1

The experiment was repeated several times using the quantities of hydrogen peroxide just necessary for decreasing the viscosity to its end value. A total of 12.0—13.6 ml N/100 NaOH were consumed in these experiments. The decrease of viscosity and the formation of titratable acid groups ceased almost simultaneously. Addition of large excess of H<sub>2</sub>O<sub>2</sub> at the end of the experiments, however, brought about a further NaOH consumption.

In another experiment the hydrogen peroxide was substituted by M/100 neutralised ascorbic acid. After 24 hours, 8.0 ml N/100 NaOH were consumed, the fall in viscosity not being complete. A blank consumed only 0.5 ml in 24 hours.

In a further experiment in which 500 mg hyaluronic acid, 0.5 ml 30 % hydrogen peroxide and 10 mg neutralised ascorbic acid were dissolved together in 50 ml water, 13.5 ml N/10 NaOH were consumed when the viscosity had reached its end value. For comparison hyaluronic acid was hydrolysed in one experiment

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We are indebted to Dr. O. SNELLMAN, Institute for Physical Chemistry, Upsala, for the double refraction measurements.

with the aid of testicular mucinase. In this case no titratable acid groups appeared. Nor were any acid products formed when the hydrolysed hyaluronic acid was treated with ascorbic acid or hydrogen peroxide.

The total amount of NaOH consumed in the experiments with hydrogen peroxide and ascorbic acid + hydrogen peroxide when the viscosity had reached its end value corresponds rather well with that required for the formation of one carboxylic group for each disaccharide unit of hyaluronic acid, constituted of N-acetylglucosamine and glucuronic acid. (The calculated value for 50 mg hyaluronic acid is 13.1 ml N/100 NaOH).

As was pointed out above the reaction products have no reducing properties and correspondingly do not give positive tests for free N-acetylglucosamine or glucuronic acid. In order to gain further information as to the nature of the products formed, the reaction mixture obtained on treating a hyaluronic acid solution with H<sub>2</sub>O<sub>2</sub> until the viscosity had reached its end value was quantitatively analysed for total glucosamine (after hydrolysis with 2N HCl) and total glucuronic acid. The values here obtained are given in Table II together with the corresponding values for the hyaluronic acid preparation used.

Table II.

Hyaluronic acid . . . . .	Glucosamine	Glucuronic acid
Before degradation . . . . .	36 %	42—46 %
After degradation . . . . .	6.5 %	38 %

Experiments with ascorbic acid gave a similar reduction of the glucosamine values. These results suggest that the main reaction product arising in these experiments is a disaccharide constituted of glucuronic acid and N-acetylglucosaminic acid. So far no attempts have been made to isolate this substance.

### Experiments on Substrates other than Hyaluronic Acid.

*Chondroitin sulphuric acid.* — This acid was prepared from cartilage without the use of alkali (BLIX and SNELLMAN, 1943). The results agreed principally with those obtained for hyaluronic acid. The fall in viscosity was, however, slower and less complete (Fig. 7).

Here also the analytical value for hexoseamine was lowered in the degraded product, although not to the same extent

as for hyaluronic acid (at most = 30 %). The glucuronic acid value did not change. Sulphuric acid was not liberated.

A few experiments were carried out with some further substrates. The viscosity of *heparin* solutions decreased somewhat when they were acted upon by ascorbic acid together with hydrogen peroxide or  $Cu^{++}$ . The same was the case with *starch solutions*. Addition of ascorbic acid to a solution of *cellulose* in Schweizer's reagent somewhat accelerated the fall in viscosity brought about by atmospheric oxygen.

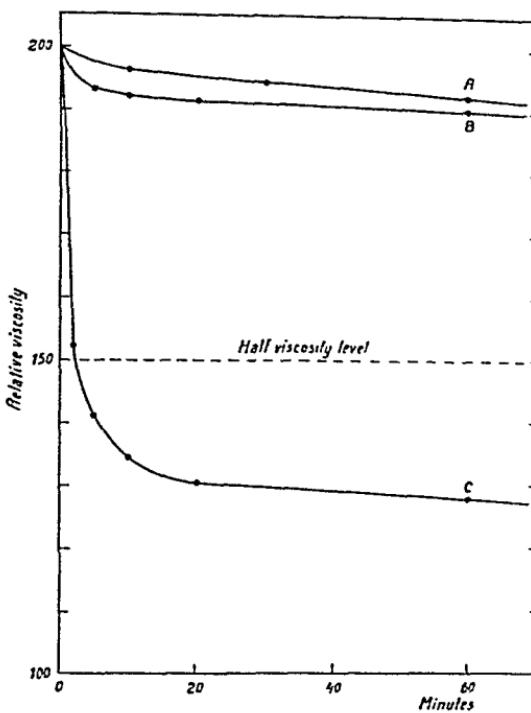


Fig. 7.

Chondroitin sulphuric acid 2 %. pH 7.2. A = 0.1 ml 3 %  $H_2O_2$  in 1 ml. solution (no ascorbic acid). B = M/100 ascorbic acid. C = M/100 ascorbic acid +  $H_2O_2$  in the same cons. as in A.

### Summary.

1. The viscosity of hyaluronic acid solutions is markedly reduced by ascorbic acid in the presence of atmospheric oxygen.  $Cu^{++}$  highly increases this effect, which is on the other hand completely inhibited by diethyldithiocarbamate or cyanide. Catalase does not inhibit the action of ascorbic acid.
2. Dehydroascorbic acid obtained by oxidation of ascorbic acid with molecular oxygen +  $Cu^{++}$  has, under aerobic conditions, the same effect as ascorbic acid +  $Cu^{++}$ .
3. The effect of ascorbic acid is also greatly increased by hydrogen peroxide, which itself although relatively slowly, reduces the viscosity of hyaluronic acid solutions. In the presence of hydrogen peroxide the ascorbic acid is also active anaerobically. The mechanism of the action of ascorbic acid is discussed.
4. A viscosity decreasing effect is brought about also by molecular oxygen (at + 100° C), potassium permanganate, and, in

the presence of atmospheric oxygen, by hydroquinone, pyrogallol and cysteine + Cu<sup>++</sup>. The effect of oxygen is inhibited by diethyl-dithiocarbamate and cyanide.

5. The products formed from hyaluronic acid during its oxidative breakdown are non-reducing and dialysable. The decrease of the viscosity is accompanied by the disappearance of the double refraction of flow shown by the native hyaluronic acid solution.

6. The oxidative depolymerisation of hyaluronic acid is connected with the formation of titratable acid groups in an amount approximately corresponding to one carboxylic group for each disaccharide unit of glucuronic acid and N-acetylglucosamine.

7. Determinations of glucosamine and glucuronic acid in the solution of the dissociated products suggest that the main reaction product might be a "disaccharide" of glucuronic acid and N-acetylglucosaminic acid.

8. Chondroitin sulphuric acid is broken down in the same way as hyaluronic acid by ascorbic acid and hydrogen peroxide. The reaction is, however, slower and does not proceed as far as that of hyaluronic acid under the experimental conditions employed.

9. Heparin, starch and cellulose could be similarly broken down to some extent by ascorbic acid.

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## On the Effect of Local Stimulation by Cold on the Adrenaline Secretion.

By

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Received 27 March 1943.

The possible rôle of the sympathico-adrenal system for the chemical heat regulation in homiothermic organisms as it is apprehended by several authors (e. g. CRAMER, 1919, CANNON, QUERIDO, BRITTON and BRIGHT, 1927) raises the question as to whether local cooling will bring about an increased activity in this system by reflex action. CRAMER (1918) found morphological changes in the suprarenal medulla of rats that had been kept at a low temperature. BOOTHBY and SANDIFORD (1923) found that injection of adrenaline increased the production of heat, and they suggested that the increased metabolism obtained, e. g. on the stimulation of the skin by cold, was due to a reflex stimulation of the adrenaline secretion.

Adrenaline seems, however, not to be necessary for the maintenance of the body temperature in cold surroundings, as is the cortical hormone (HOUSSAY, LEWIS, 1923, HARTMANN, BROWNELL, CROSBY, 1931, and WYMAN and TUM SUDEN, 1932).

A number of writers have shown that adrenaline secretion increases as a result of stimulus by cold. HARTMANN, MCCORDOCK and LODER (1923), in experiments on the cat, employed the increase in width of a totally denervated pupil as a biological test for increased adrenaline secretion. They found that if the animals were exposed to cold, the width of the denervated pupil increased. Under the same conditions epinephrectomized animals showed no or only slight dilatation of the pupil. In experiments on cats with

denervated hearts as test animals, CANNON, QUERIDO, BRITTON and BRIGHT (1927) have confirmed the increased adrenaline secretion. In experiments on cats with totally denervated pupils, HARTMANN and HARTMANN (1923) showed it to be probable that the increased adrenaline secretion is due rather to a reflex influence of the suprarenals through the peripheral cold-stimulus than to a lowering of the general body temperature.

In the present paper the results of studies on the effect on the adrenaline secretion of local stimulation with cold, as well as the connection of this effect with the nervous supply to the stimulated skin region are reported. The increase in width of a sympathetically denervated pupil has been employed as indication of an increased secretion. In order to control whether a dilatation was due to adrenaline, we used injections of cocaine, which is known to increase the action of adrenaline (FROELICH and LOEWI, 1910), with a fair degree of specificity.

### Methods.

The experimental objects used were white rabbits, weighing between two and three kilograms. In these animals one pupil was made over-sensitive to adrenaline by extirpation of the superior cervical ganglion according to the method described by MELTZER and MELTZER-AUER (1904), while the other pupil was left intact. The increase in the width of the denervated pupil has been used as a biological indication of increased adrenaline secretion, the other pupil serving as a control. In every animal the over-sensitiveness to adrenaline of the denervated pupil was controlled by the intravenous injection of 10—20 µg adrenaline. Only the animals showing a clear over-sensitiveness to these doses were employed in the later experiments with local stimulation by cold, which were carried out 2—4 hours after the test for over-sensitiveness, when the adrenaline effect had completely disappeared.

The local stimulation by cold was carried out on a skin area of 80—100 cm<sup>2</sup> on the belly. The cooling was effected both on animals with intact skin innervation and on animals in which the innervation to the stimulated skin area had been cut off. The skin-denervation of a number of the animals was performed according to the method described by von REIS and SJÖSTRAND (1938), which entails the severing of the lateral branches from the lowest intercostal and superior lumbar nerves, in certain cases in the following way. From a longitudinal cut in the medial line of the back, the skin is bluntly dissected off from the animal's trunk. Since, in the case of rabbits, the skin of the trunk is mainly supplied from the vessels of the extremities, the operation causes only slight bleeding. With this method one obtains

a complete denervation for the stimulation by cold of a sufficiently large skin area on the trunk.

The local stimulation by cold was effected with the help of a thin bag of oiled cloth containing a mixture of ice and salt. The temperatures between the cold bag and the shaved skin varied between 0 and + 10° C.

The experiments were carried out both on non-narcotized animals and on animals in pernocton narcosis (0.5 cc/kg body weight). Illumination was kept constant and the same on both eyes. The width of the pupils was determined with the help of a sliding gauge, with which it was possible to obtain measurements with a limit of error of about 0.5 mm.

The cold-stimulation was begun after the width of the pupil had become constant when the animal was lying on its back. The stimulation was maintained for a period of one to two hours, during which the width of the pupils, as well as rectal and skin temperatures, were measured at regular intervals. In a number of experiments 0.2 mg cocaini hydrochloridum was given intravenously at the end of the cold-stimulation.

On a number of animals with intact innervation of the skin cold-stimulation was effected after lumbar anaesthesia (1 ml 2 % aethocaini hydrochloridum). On two of the animals the spinal cord was severed at the level of Th 2; cold-stimulation was then effected on these animals about four days after the operation.

## Results.

The 8 animals with intact skin innervation, on which a local cold-stimulation was effected, all showed an increase in the width of the denervated pupil. The increase amounted to between 1.5 and 4.5 mm, on an average 3.0 mm. The width of the intact pupil increased by between 0 and 1 mm, on an average 0.5 mm (see table). Thus in all cases we find an increased adrenaline secretion. Fig. 1 is an example of the changes in pupil width and rectal temperature in one of the animals.

Fig. 2 shows the changes in the width of the pupil in an animal to which, after an increase of 3.5 mm in the width of the denervated pupil owing to cold-stimulation, 0.2 mg of cocaine was given. This rapidly led to a further dilatation, amounting to 3.5 mm. The width of the intact pupil had increased, during the stimulation by cold, by 1 mm, and after the injection of cocaine it showed a further increase of 1 mm. Thus the total increase in the width of the denervated pupil was 7 mm, while the intact pupil showed a corresponding increase of only 2 mm. The additional increase in width of the denervated pupil that was observed

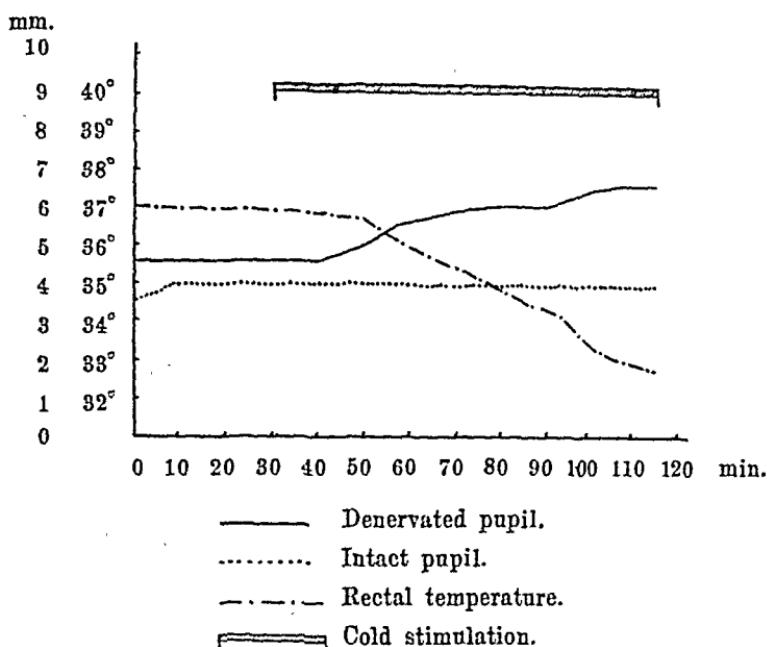


Fig. 1. Rabbit, left pupil sympathetically denervated.

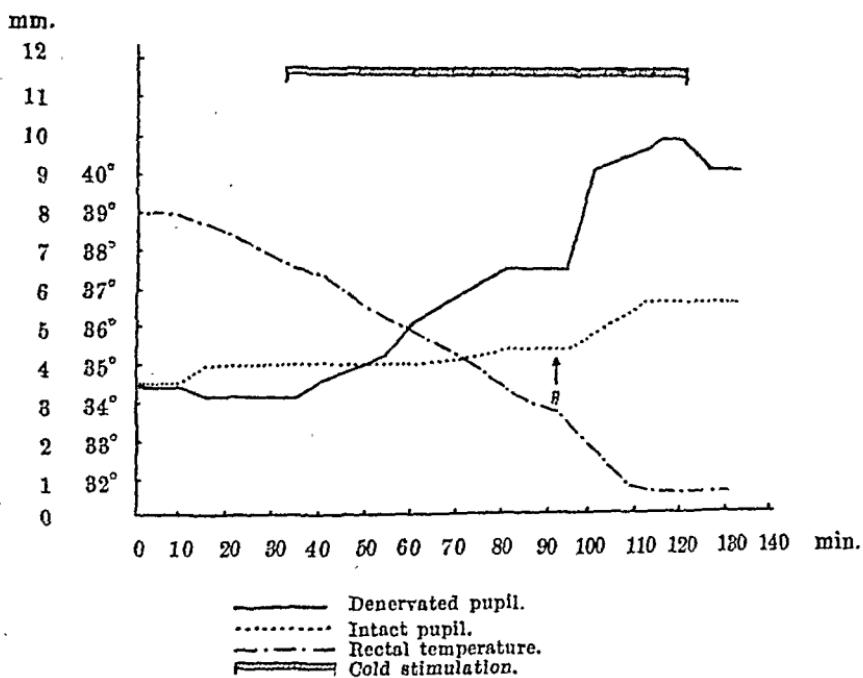


Fig. 2. Rabbit, left pupil sympathetically denervated. At A intravenous injection of 0.2 mg cocaini hydrochloridum.

Table.

	Pupil-width (increase in mm)				Lowering of rectal tempera- ture °C.	Cooling tempera- ture (average) °C.		
	Intact pupil (right)		Denervated pupil (left)					
	A	B	A	B				
A. Animals with intact skin innervation.								
1 . . . . .	0.0	—	1.5	—	1.2	8.5		
2 . . . . .	0.5	—	2.5	—	6.0	3.0		
3 . . . . .	1.0	—	4.5	—	10.0	3.0		
4 . . . . .	0.5	—	3.0	—	4.0	4.0		
5 . . . . .	0.5	—	3.0	—	1.5	9.0		
6 . . . . .	1.0	1.0 (2.0)	3.5	3.5 (7.0)	5.0	2.5		
7 . . . . .	0.0	0.5 (0.5)	2.0	2.5 (4.5)	4.8	1.0		
8 . . . . .	0.5	1.0 (1.5)	4.0	3.0 (7.0)	3.3	3.0		
	Average	0.5	0.8 (1.8)	3.0	3.0 (6.2)	4.5		
9 . . . . .	0.0	0.0 (0.0)	2.0	2.0 (4.0)	0.0	6.0		
10 . . . . .	0.5	0.5 (1.0)	2.5	3.0 (5.5)	0.0	3.0		
11 . . . . .	1.0	0.0 (1.0)	2.5	3.5 (6.0)	0.0	4.0		
	Average	0.5	0.2 (0.7)	2.8	2.8 (5.1)	0.0		
Narcotized animals (0.5 cc pernocton/kg. body-weight).								
12 . . . . .	0.5	—	3.0	—	4.5	5.0		
13 . . . . .	1.0	—	3.0	—	4.0	8.0		
14 . . . . .	0.0	—	3.0	—	3.2	0.0		
15 . . . . .	0.0	1.0 (1.0)	2.0	3.0 (5.0)	5.0	2.0		
16 . . . . .	1.0	1.0 (2.0)	2.5	2.5 (5.0)	0.0	3.5		
	Average	0.5	1.0 (1.5)	2.7	2.8 (5.0)	3.3		
Narcotized animals. Cold stimulation in lumbar anaesthesia (1 cc 2 % aethocaini hydrochloridum).								
17 . . . . .	— 0.5	—	— 1.0	—	6.0	3.5		
18 . . . . .	— 0.5	—	— 1.0	—	3.8	6.0		
19 . . . . .	0.0	—	0.0	—	5.8	1.0		
	Average	— 0.5	—	— 0.7	—	5.2		
						3.5		

	Pupil-width (increase in mm)				Lowering of rectal tempera- ture °C.	Cooling tempera- ture (average) °C.		
	Intact pupil (right)		Denervated pupil (left)					
	A	B	A	B				
Animals with spinal cord severed.								
20 . . . . .	- 0.5	0.0 (- 0.5)	0.0	0.5 (0.5)	4.2	2.5		
21 . . . . .	- 0.5	0.0 (- 0.5)	0.5	0.5 (1.0)	5.0	3.0		
Average	- 0.5	0.0 (- 0.5)	0.3	0.5 (0.8)	4.6	2.8		
B. Animals with stimulated skin-area denervated.								
22 . . . . .	1.0	—	- 0.5	—	5.4	0.0		
23 . . . . .	1.5	—	0.0	—	4.7	10.0		
24 . . . . .	0.0	—	0.0	—	2.7	6.0		
25 . . . . .	1.0	0.5 (1.5)	0.5	1.0 (1.5)	5.5	2.0		
26 . . . . .	1.5	0.5 (2.0)	1.0	1.0 (2.0)	1.8	8.0		
27 . . . . .	1.0	1.0 (2.0)	0.5	1.5 (2.0)	0.0	3.0		
Average	1.0	0.7 (1.8)	0.3	1.2 (1.8)	3.4	4.8		

A = the increase in width caused by the cold-stimulation.

B = additional increase caused by injection of cocaine.

In brackets the sum: dilatation by cold + additional increase by cocaine.

after the injection of cocaine, makes it highly probable that the dilatation was due to an adrenaline effect.

On 5 animals the cold-stimulation was effected under pernocton narcosis. The results obtained from these experiments show full agreement with the above-mentioned figures with animals in the conscious state.

Local cold-stimulation was effected on 6 animals with a denervated skin area. In these animals the denervated pupil showed a change varying between - 0.5 and 1.0 mm, on an average an increase of 0.3 mm. The other pupil showed an increase in width varying between 0.5 and 1.5 mm, on an average 1.0 mm. These slight increases in width fall within the normal limits of variation. Especially the denervated pupil showed constant values. We found no demonstrable adrenaline secretion resulting on the stimulation by cold of animals with denervated belly skin, despite the fact that the animals were submitted to a very severe general

cooling (see the table). Fig. 3 shows the pupil width and the rectal temperature during the cold-stimulation of one of the animals.

When 0.2 mg of cocaine was given during the cold-stimulation on an animal with denervated belly skin, the denervated pupil increased by 1 and the other pupil by 0.5 mm (Fig. 4). These values lie within the limits for the pupil changes that are normally

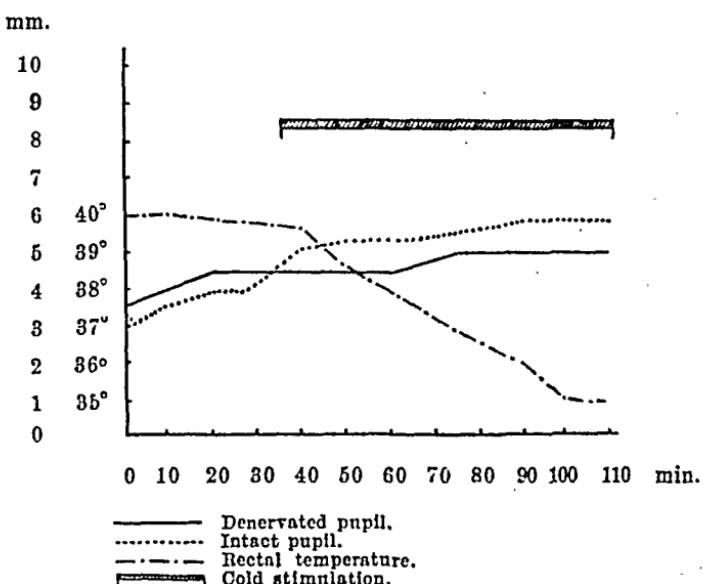


Fig. 3. Rabbit, left pupil sympathetically denervated, cold-stimulated skin area denervated.

obtained on the injection of cocaine, and they cannot be said to constitute an intensification of a possible adrenaline effect.

In some experiments the observations of the pupil width and the rectal temperature were continued after the completion of the cold-stimulation, and these showed a return to normal size of both the intact and the denervated pupil, although the rectal temperature fell still further. These experiments afford additional evidence that the increased adrenaline secretion is due to a peripheral stimulation effect, and that a fall of the general body temperature does not lead to any increase in the adrenaline secretion that is demonstrable by this method.

Cooling experiments were also carried out on rabbits with intact belly skin innervation, special arrangements being employed ("Hala"-lamp, wadding) to keep the general body temperature constant during the whole experiment. The pupils showed the

same relations as in the above-mentioned experiments, where the body temperature was allowed to fall (see the table). The nervous reflex action on the adrenaline secretion is thus independant of whether the general body temperature falls below normal or not.

In the table are also given some experiments on animals whose spinal cord was severed on a level with Th 2, and also on

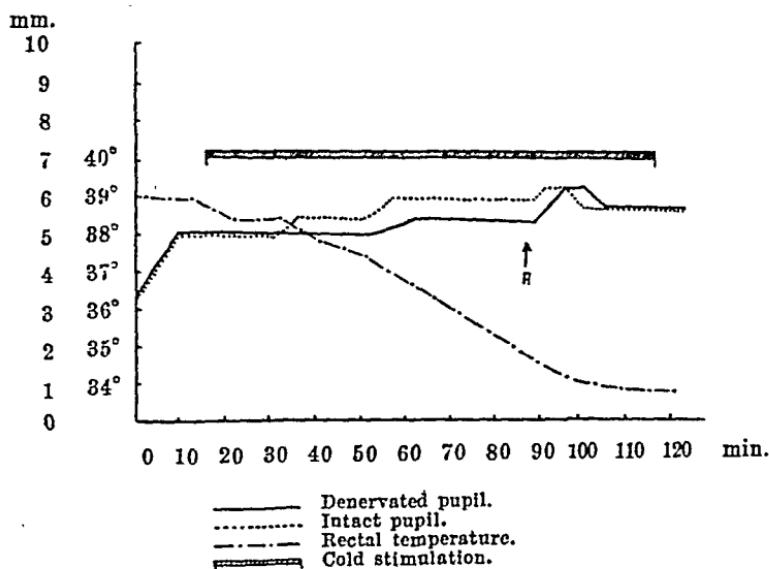


Fig. 4. Rabbit, same as in fig. 3. At A intravenous injection of 0.2 mg cocain hydrochloridium.

animals on which lumbar anaesthesia had been performed. In both cases no increase in the width of the denervated pupil was observed, despite the intact skin innervation.

### Discussion.

As an indication of the increased adrenaline secretion resulting on local cold-stimulation, we have studied the increase in width of a sympathetically denervated pupil, which was found to attain its maximum of over-sensitiveness to adrenaline at the earliest five days after the operation.

A dilatation of the pupil can also follow on an inhibition of *musculus sphincter pupillae*. Since, in the experiments described in this paper, the width of the intact pupil remained practically

constant, such inhibition may here be excluded. The further dilatation of the denervated pupil after cocaine had been given intravenously, strongly suggests that the effect was due to an increased content of adrenaline or of some closely allied derivative in the blood. In the animals on which cold-stimulation was effected within a denervated skin area, without any primary pupil dilatation being obtained, the cocaine injection gave no or only very slight effect, not exceeding the effect on the non-denervated pupil.

The fall of the rectal temperature in animals with intact and denervated belly skin was on the whole in agreement. The fall in temperature amounted in general to about  $4^{\circ}$  C., and was thus very considerable. An increased adrenaline secretion, however, could only be demonstrated in animals in which the innervation of the skin exposed to cooling was intact. From this it appears that the increase in the adrenaline secretion is due to a reflex action. Another important conclusion can also be drawn from these results, namely that a sinking of the general body temperature has no effect on the adrenaline secretion that can be demonstrated by this method. This is in conformity with the view, held by JOHANSSON (1897), that there is no chemical temperature regulation when muscle movements are abolished, and that a change in the body temperature *per se* does not give rise to the chemical regulation. This is further supported by the experiments in which, after the completion of the cold-stimulation, the pupil with returned to the original value, although the rectal temperature continued to sink to a minimum value of about  $32.8^{\circ}$  C.

That the adrenaline secretion has the character of a nervous reflex is also shown by the fact that it completely disappears after lumbar anaesthesia or after the spinal cord has been severed.

### Summary.

1. The adrenaline secretion following on local cold-stimulation of the skin has been studied in the rabbit. As an indication of increased adrenaline secretion, the increase in width of a sympathetically denervated pupil, sensitized by cocaine, was employed.
2. In animals with intact innervation of the skin the adrenaline

secretion showed an increase during the cold-stimulation, even if body temperature was kept constant. This effect was not produced after previous section of the spinal cord or after lumbar anaesthesia.

3. In animals in which the innervation to the cold-stimulated skin area had been cut off, no demonstrable increase in the adrenaline secretion appeared in response to the stimulation. The effect of the cold-stimulation on the adrenaline secretion is thus reflex in kind.

4. Even a considerable fall in general body temperature did not in itself provoke any detectable reflex liberation of adrenaline.

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## On the Glucosidases of the Intestine of the Pig.

By

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Received 9 April 1943.

By the efforts of numerous workers a large amount of experimental material has been accumulated concerning the glucosidases from vegetable sources. As regards the glucosidases of animal origin, much less is known. Thus, it may be mentioned that these enzymes occur in liver and kidney, and also in the intestinal mucosa. As regards this latter organ, existing evidence is rather conflicting in certain respects (MYRBÄCK and MYRBÄCK 1936, WEIDENHAGEN 1940), and we have therefore thought it worth while to undertake a renewed investigation of it.

The method employed was the very familiar one of working with glycerol or aqueous extracts from the organ, and studying polarimetrically the enzymic hydrolysis of suitable glucosides. We have thereby found it advantageous to work with aqueous extracts. The results are as follows (for numerical details see below). The intestinal mucosa contains an  $\alpha$ - as well as a  $\beta$ -glucosidase. The  $\alpha$ -glucosidase exhibits on the whole the same properties as the corresponding enzyme in yeast. It has a pH optimum around 6.5 (in a phosphate-citrate buffer). Furthermore, it hydrolyses phenol- $\alpha$ -d-glucoside incomparably more rapidly than it does methyl- $\alpha$ -d-glucoside. In fact, we were unable to find any measurable hydrolysis of this latter compound, even in experiments of long duration. We are thus able to confirm completely the findings of MYRBÄCK and MYRBÄCK (1936) on this point. Finally it may be mentioned that we have found our aqueous extracts remarkably stable, while  $\alpha$ -glucosidase preparations from yeast are known to lose their activity rather rapidly (after a few days).

The  $\beta$ -glucosidase from the small intestine does not show any unexpected behaviour either. We found a pH optimum around 6.0 (in phosphate-citrate buffer). The  $\beta$ -glucosidase from bitter almonds has its optimum around 4.4; but it may be mentioned that the corresponding enzymes from kidney and liver, and from certain vegetable sources, are likewise known to have their optima around 6.0. We have, however, found an interesting difference in behaviour between the  $\beta$ -glucosidase from the small intestine and that from bitter almonds. The latter enzyme is known to hydrolyse o-cresol- $\beta$ -d-glucoside much more rapidly than phenol- $\beta$ -d-glucoside (relation between the velocity constants ca. 13: 1), while we found for the former enzyme a relation between the velocity constants of about 2 : 1. However, the crudity of our enzymic extracts must be born in mind, and refined experiments, with purified enzymic extracts, may well show the effect to be due to impurities. We do think, however, that the possibility of a difference between the two enzymes should be kept in mind by future workers on the subject.

We have also investigated aqueous extracts from the duodenum, and found them to contain both  $\alpha$ - and  $\beta$ -glucosidase. The velocity constants for the latter enzyme with o-cresol- $\beta$ -d-glucoside as substrate showed a very peculiar behaviour, the constants increasing greatly with time during the experiment. We have no definite explanation to offer for this curious phenomenon, but are inclined to ascribe it to some impurity in our extracts.

### Experimental Part.

*Enzymic extract.* A piece of intestine, ca. 50 cm. in length, was carefully emptied of its contents in running water, and the mucosa scraped off, mixed with sea sand and carefully ground in a mortar. The mixture was then extracted with around 200 cm<sup>3</sup> of water for several hours and left standing in the ice-box overnight: finally it was centrifuged. Some extracts were prepared using 200 cm<sup>3</sup> glycerol, but they were found to be much less active than the aqueous extracts and all the work to be described below was therefore performed with the latter ones. These solutions, with some drops of toluene added for conservation purposes, were found to preserve their activity unaltered over a period of around 3 weeks.

*Substrates.* The glucosides used here were synthesised by current methods, which do not need to be described in detail; they are to be found in most of the newer reports on the subject.

*Measurements.* After some preliminary tests, undertaken to get a

rough idea of pH optima and reaction velocities in the systems under consideration, the following experiments were carried out.

We have used throughout McIlvain's phosphate-citrate buffer, by means of which the required pH interval can be easily covered. A quantity of glucoside, chosen so as to make the reaction mixture 0.04 molar was dissolved in 6 cm<sup>3</sup> buffer solution; then 10 cm<sup>3</sup> enzymic extract was added together with a quantity of water so that the total volume of the reaction mixture was 25 cm<sup>3</sup>. The reaction vessels were placed in an incubator at 30° C. At suitable time intervals a test sample of 5 cm was removed by means of a pipette and added to 1 cm<sup>3</sup> of 20 per cent potassium carbonate solution. After two hours (to avoid errors due to mutarotation), the solution was treated with kieselgur and filtered through kieselgur impregnated paper. After this treatment the solution was clear enough to allow of a polarimetrical measurement, which was carried out in a 10 cm tube. The initial rotations for the glucosides used were under these conditions:

Methyl- $\alpha$ -d-glucoside . . .	+ 1.02°	Phenol- $\beta$ -d-glucoside . — 0.63°
Phenol- $\alpha$ -d-glucoside . . .	+ 1.535°	$\alpha$ -Cresol- $\beta$ -d-glucoside . — 0.66°

The final rotation after complete hydrolysis of the glucosides was in all cases + 0.310°. Attention has been paid to the influence of the pH on the rotation of the  $\beta$ -glucosides and glucose; a similar influence was not found for the  $\alpha$ -glucosides here examined.

In separate experiments it was shown that the self-hydrolysis of the glucosides was unimportant and could be left out of consideration, and that the rotation of the enzymic solution was likewise negligible.

Table 1 gives the results of the investigation of methyl- $\alpha$ -d-glucoside. As a check phenol- $\beta$ -d-glucoside was measured at the same time. In our tables  $\alpha$  denotes the observed rotation and  $k$  the first order velocity constant.

Table 1.

Time (hours)	(Phenol $\alpha$ -d-glucoside) $\alpha$	(Methyl-d-glucoside) $\alpha$
45 . . . .	—	1.01
93 . . . .	1.32	1.02
139 . . . .	1.12	1.02
188 . . . .	0.92	1.02

Tables 2 and 3 give the results of measurements undertaken to obtain pH-acitivity curves for the  $\alpha$ - and  $\beta$ -glucosidases of the small intestine.

Table 2.  
*Phenol- $\alpha$ -d-glucoside.*

Time (hours)	pH = 3			4			5			6			7			8		
	$\alpha$	$k$	$\alpha$	$k$														
23 . . . . .	1.50	$0.63.10^{-3}$	1.51	$0.47.10^{-3}$	1.48	$0.94.10^{-3}$	1.44	$1.60.10^{-3}$	1.44	$1.60.10^{-3}$	1.48	$0.94.10^{-3}$	1.48	$0.94.10^{-3}$	1.44	$0.84$		
44 . . . . .	1.48	0.49	1.47	0.57	1.43	0.92	1.38	1.38	1.37	1.47	1.44	1.44	1.44	1.44	1.38	0.89		
68 . . . . .	1.42	0.60	1.41	0.71	1.35	1.07	1.29	1.45	1.29	1.45	1.38	1.38	1.38	1.38	1.32	0.93		
92 . . . . .	1.37	0.70	1.34	0.83	1.28	1.11	1.18	1.63	1.17	1.68	1.17	1.68	1.17	1.68	1.32	0.93		

Table 3.  
*o-Cresol- $\beta$ -d-glucoside.*

Time (hours)	pH = 3			4			5			6			7			8		
	$\alpha$	$k$	$\alpha$	$k$														
19 . . . . .	0.61	$1.23.10^{-3}$	0.57	$2.23.10^{-3}$	0.55	$2.75.10^{-3}$	0.50	$4.12.10^{-3}$	0.55	$2.75.10^{-3}$	0.61	$1.21.10^{-3}$	0.61	$1.21.10^{-3}$	0.56	$1.13$		
42 . . . . .	0.58	(0.89)	0.49	1.99	0.42	2.94	0.36	3.88	0.43	2.80	0.30	3.01	0.30	3.01	0.56	1.13		
67 . . . . .	0.56	(0.71)	0.41	1.93	0.30	3.01	0.20	4.17	0.30	3.01	0.56	1.17	0.56	1.17	0.56	1.17		

In Table 4 are collected the results of measurements undertaken to compare the hydrolysis of phenol- $\beta$ -d-glucoside and o-cresol- $\beta$ -d-glucoside.

Table 4.

Time (hours)	Phenol- $\beta$ -d-glucoside (pH = 6)		o-Cresol- $\beta$ -d-glucoside (pH = 6)	
	$\alpha$	k	$\alpha$	k
21 . . . .	- 0.55	$1.84 \cdot 10^{-3}$	- 0.51	$3.48 \cdot 10^{-3}$
45 . . . .	- 0.43	2.31	- 0.40	3.01
71 . . . .	- 0.30	2.65	- 0.29	2.94

Tables 1 and 4 are obtained with enzymic material from one and the same animal, and Tables 2 and 3 with material from another. A piece of duodenum was taken from a third animal and the results obtained with it are shown in Table 5. A repetition of this experiment gave essentially the same result.

Table 5.

Phenol- $\alpha$ -d-glucoside (pH = 6)			o-Cresol- $\beta$ -d-glucoside (pH = 6)		
Time (hours)	$\alpha$	k	Time (hours)	$\alpha$	k
22 . . . .	- 1.41	$2.20 \cdot 10^{-3}$	21 . . . .	- 0.50	$3.73 \cdot 10^{-3}$
45 . . . .	- 1.31	2.00	45 . . . .	- 0.29	4.64
69 . . . .	- 1.20	2.04	68 . . . .	- 0.10	5.50
95 . . . .	- 1.09	2.08	92 . . . .	+ 0.09	7.04

### Summary.

An investigation is made of the glucosidases of the small intestine and the duodenum of the pig. It is shown that both  $\alpha$ - and  $\beta$ -glucosidase occur, and the pH-activity curves of both enzymes are determined.

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## Studies in Acute but Moderate CO-Poisoning.

By

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Received 12 April 1943.

The effect of mild to moderate acute carbon monoxide poisoning on the respiration and the circulation has recently been studied by ASMUSSEN and CHIODI (1941) and by CHIODI, DILL, CONSO LAZIO and HORVATH (1941). The main results were, that the respiration was unaffected up to rather severe poisonings, and that the cardiac output was practically unchanged up to HbCO saturations of about 30 pCt., whereas more severe poisonings caused a moderate increase (20—50 pCt.) of the cardiac output. In neither case, however, the circulation was able to maintain a normal capillary or tissue  $O_2$ -pressure, but this would be abnormally low, partly because of the decreased  $O_2$ -binding capacity of the arterial blood, and partly because the  $O_2$ -dissociation curve of the available hemoglobin is shifted to the left (STADIE and MARTIN 1925).

It might be of interest to investigate whether such a reduction of the capillary or tissue  $pO_2$  influences other functions of the organism. Similar investigations have been made before (cf. the extensive bibliography in DRINKER 1938) but, as it appears, mostly on animals and during excessive CO-poisonings. Observations on man in CO-poisoning are to a large degree limited to clinical observations made during severe asphyxiations. HALDANE (1895), however, besides making fundamental explorations on the effect of CO on the blood, made interesting observations on the mental ability during CO-poisoning as later did FORBES, DILL, DE SILVA and VAN DEVENTER (1937), SJÖSTRAND (1942) a. o., and KILICK (1936) has made extensive studies in the acclimatiza-

Table

Subject		Cell volumes pCt.			
		before CO	after CO	control I	control II
E. A. . . . .	pCt. . . . .	41.9	43.6	41.5	41.9
	range . . . . .	40.8—43.0	40.6—45.2	40.7—42.8	41.4—43.7
	nbr. of determ.	12	13	10	10
E. Kn. . . . .	pCt. . . . .	41.1	43.5	40.2	41.1
	range . . . . .	38.3—42.4	41.6—45.2	38.4—41.8	40.0—41.8
	nbr. of determ.	8	9	8	6

tion of humans to CO in low concentrations. We have chosen to study the effect of moderate CO-poisonings, viz. poisonings with about 30 pCt of the hemoglobin saturated with CO. At this degree of poisoning the subjective symptoms, apart from the head-ache, are almost negligible, although the tissue  $pO_2$  is already considerably lowered ( $pO_2$  of mixed venous blood lowered from the normal about 37 mm Hg to about 23 mm Hg, ASMUSSEN 1942). In daily life these mild and moderate degrees of poisoning are by far the most common.

Among the functions studied were: the relative erythrocyte volume, the plasma protein concentration, the blood sugar and the blood lactate in rest and during moderate work. The function of the intestine was tested by water drinking experiments and by glucose tolerance tests, which also give informations concerning the functioning of the kidneys and of some of the endocrine glands. In order to elucidate the findings on the blood, the increase in capillary filtration was determined on a lower extremity when the hydrostatic blood pressure was increased, and in an attempt to evaluate the peripheral distribution of the blood during CO-poisoning, the vital capacity was determined.

*Subjects and methods:* We ourselves served as subjects. E. A. male, is 35 years, weight 70 kg., height 172 cm, E. Kn. male, is 30 years, weight 67 kg, height 185 cm. Both were in healthy condition during the experimental time. The CO was prepared from formic acid and sulphuric acid, bubbled through a solution of NaOH and stored over water. 400 cc of CO at prevailing temp. and barometric pressure were introduced into a KROGH metabolism apparatus, filled afterwards with  $O_2$ , and rebreathed for 10—15 minutes. The actual experiments were performed during the recovery period following. Blood samples,

## I.

Plasma proteins pCt.			
before CO	after CO	control I	control II
7.32	7.59	7.32	7.38
7.00—7.56	6.91—8.00	7.00—7.55	7.13—7.59
9	13	6	6
7.45	7.64	7.35	7.25
7.35—7.54	7.42—7.89	6.85—7.80	6.86—7.72
8	9	8	6

drawn from the cubital vein just before and just after the experiment and analysed after VAN SLYKE and NEILL 1924 for CO, gave the ranges between which the blood HbCO had moved during the experiment. The elimination of CO was rather slow, in rest the percentage HbCO fell about 5 in an hour (e. g. from 32 pCt HbCO to 27 pCt HbCO). The cell volume of the blood was read from hematocrits, small glass tubes, closed in one end and taking about 0.5 cc of venous blood, centrifuged at 3000 rev/min. for 30 minutes. Heparin was used as an anticoagulant. The plasma proteins were determined by a Zeiss "Eintauch Refraktometer" on the plasma from the hematocrits. Blood sugars were determined according to HAGEDORN and JENSEN (1941) and blood lactates after EDWARDS (1938). An estimation of the rate of filtration through the capillary walls was made by determining the increase in volume of one leg placed in an airfilled plethysmograph in connexion with a small spirometer, when the subject was tilted passively from the horizontal position to a position with the head upwards at an angle of 45° (hereafter called the + 45° position). In this position a rapid increase in the volume of the leg occurs, followed by a slow, but consistent swelling. The rapid increase is due to the filling-up of the vessels with blood, and lasts only a couple of minutes. The progressive swelling of the leg is caused by the leaking-out of fluid from the capillaries and goes on at a fairly constant rate for up to an hour or more (comp. ASMUSSEN, CHRISTENSEN and NIELSEN, 1939). We used the swelling of the leg (from the 2. to the 7. minute) as a measure of the filtration, and express it in cc/min.

Parallel to the CO experiments we ran control experiments in order to be sure that the observed changes were actually due to the CO-poisoning.

*Results:* The results from our determinations on the blood cell volume and the plasma proteins are presented in table I. The heads "control I" and "control II" refer to determinations made, respectively, in the first and in the second hour of the control experiments. "Before CO" are values obtained in the hour

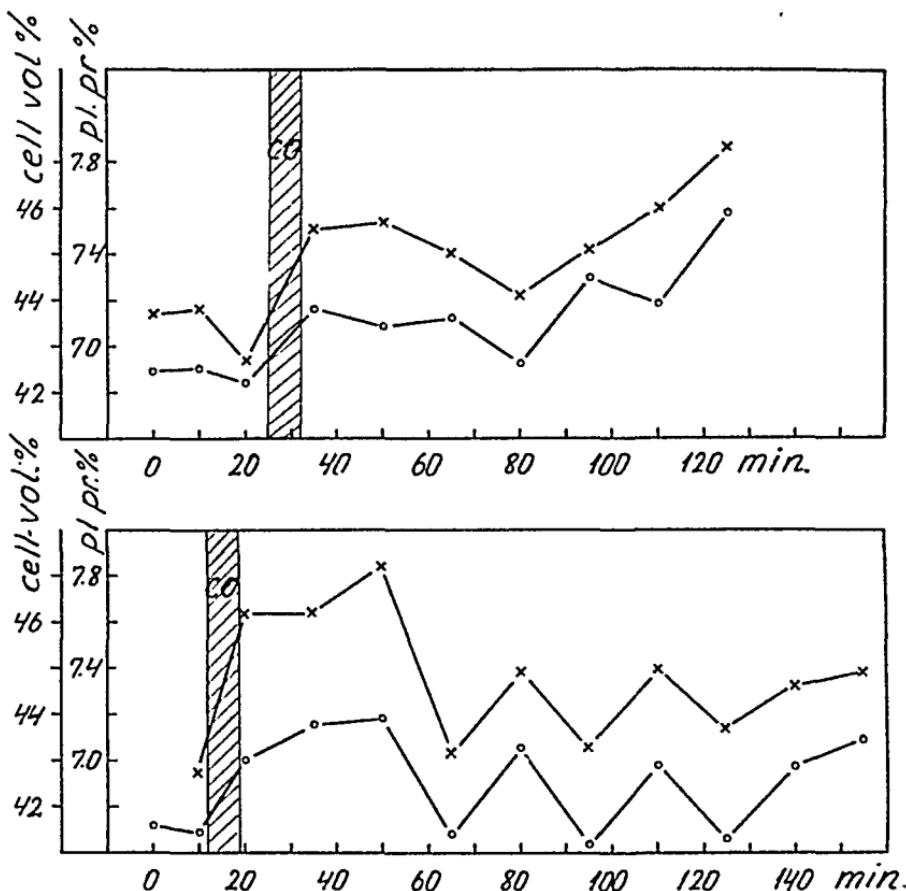


Fig. 1. Two experiments, showing the effect of CO-poisoning on the blood. Subj. E. A.  $\times$ — $\times$  plasma protein concentration;  $\circ$ — $\circ$  red cell volume; dark column indicates time of CO-inhalation.

immediately before the CO was inhaled, and "after CO" refers to values obtained during the first hour after the inhalation of CO. The hemoglobin of the blood was 30—32 pCt. saturated with CO five minutes after cessation of the inhalation and at the end of the following hour 25—27 pCt saturated. The average HbCO pCt thus was 27—29.

Table I shows, that both the cell volume and the plasma protein concentration on an average are increased in the first hour after a moderate CO-intoxication. The rather wide range, inside which the individual values are scattered, may be explained through the fact, that the subjects were not under strictly standard conditions.

In order to indicate when the observed hemoconcentration occurs, we present fig. 1. It shows, that already 10 to 15 minutes

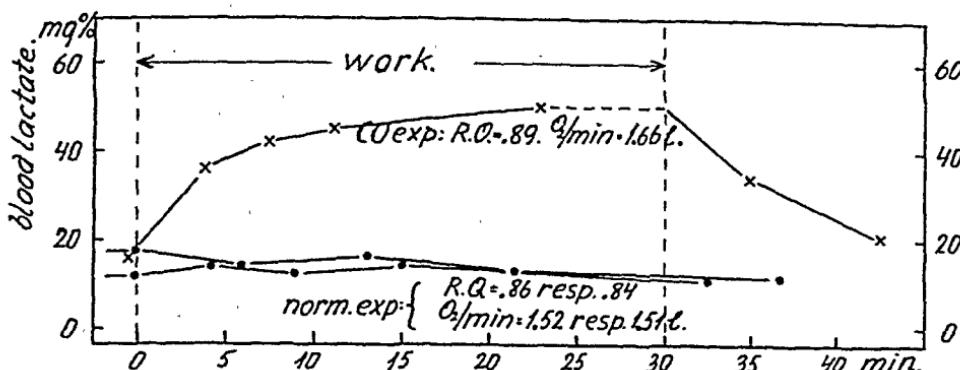


Fig. 2. Blood lactate during and after work of 720 mkg/min. Subj. E. A.  
 ●—● normal experiments; x—x after CO-inhalation.

after the CO-inhalation has started, the cell volume and the plasma protein concentration are increased. About an hour later the values again approach the normal values, but then a second, increase seems to start. In all of the five experiments, in which we followed the blood changes for more than one hour, we found this secondary rise in hemoconcentration. In some cases — as that presented in fig. 1 upper curves — the secondary rise was, for so long as it was followed, fairly steady. In one case (fig. 1, lower curves) however, in which the blood changes were followed for more than two hours after the CO-inhalation, the secondary rise was followed by a period during which both the cell volume and the plasma protein concentration made rather large but parallel fluctuations.

Our observations on the blood sugar and the blood lactate during moderate CO-poisoning can be shortly dealt with, as they showed, that neither were affected as long as the subjects were resting. Two experiments on E. A. during exercise (modified KROGH bicycle ergometer, working intensity 720 mkg/min.) showed, however, that under conditions of work the blood lactate was increased, whereas the blood sugar was still unaffected. In Fig. 2 are presented data from an experiment with from 30—25 pCt. HbCO, compared with two normal experiments. The figure shows, that whereas in the normal condition this rate of work has no influence on the blood lactate, a HbCO percent of 30—25 makes the blood lactate increase up to 50 mg%. Determinations of the metabolic rate and the RQ during the steady state of work (Douglas bag method) gave as a result, that both the O<sub>2</sub>-intake and RQ were higher when the blood contained HbCO enough to give an

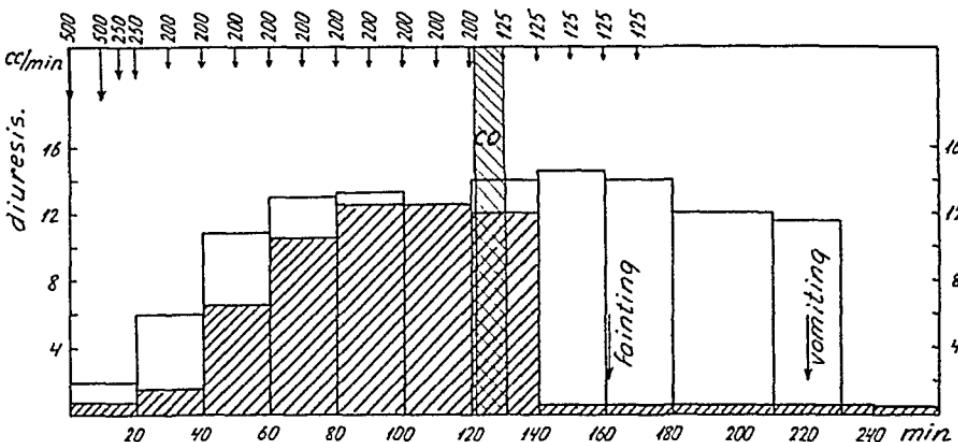


Fig. 3. Water drinking experiments on subj. E. Kn. Figures in top indicate amount of water drunk. White columns: diuresis in normal experiment, dark columns; diuresis in CO-experiment. CO inhaled between 120. and 130. minute.

increased lactate formation. The data given in fig. 2 are averaged from three single determinations each, made in the steady state of work.

In order to investigate the function of the intestine during CO-poisoning, a water drinking test was performed in the same way as used earlier by ASMUSSEN, CHRISTENSEN and NIELSEN (1939) to determine the effect of posture on the intestinal tract. The subject drank in the horizontal position 200 cc of water every 10 minutes until the diuresis became constant. The CO was then administered in the usual way after which the waterdrinking continued, the water intake now being adjusted so as to equal the output.

The results came out differently for the two subjects: in E. A., whose diuresis was raised to and kept constant at 20 cc/min., the inhalation of 400 cc of CO (31 pCt. HbCO) had no effect on the absorption or excretion of water over a period of more than two hours. (At the end of this period the HbCO had sunk to 24 pCt.). In E. Kn. (fig. 3) the diuresis rose to 13.5 cc/min. and the water intake was adjusted to this output. The CO was then given (32 pCt. HbCO) and the water drinking continued. In the first 20 minute period thereafter the diuresis was still 13.5 cc/min, but then, after an attack of nausea and a faint spell on assuming the standing position, the diuresis suddenly dropped to a very low level (0.5 cc/min.) for the next almost 2 hours. The water drinking was suspended shortly after the faint spell, the subject placed with elevated feet, and a CO<sub>2</sub>-O<sub>2</sub> mixture administered. Close to the

end of this period 600 cc of water were thrown up. Later in the evening (i. e. approximately 5 hours after the CO-inhalation) again about 500 cc of water were thrown up. The diuresis remained low until late in the night, when the rest of the water was excreted along the natural way.

On account of the unpleasantness to the subject, this experiment was not repeated, but two days later a control experiment without CO inhalation was started, during which the subject drank the same amount of water, this time without bad effects of any kind. (fig. 3, white columns).

As it might be expected that the absorption of sugar from the intestine was more dependent on an adequate O<sub>2</sub>-supply to the cells in the intestinal wall than is the absorption of water (comp. the theory of LUNDSGAARD and co-workers, (lit. by KJERULF-JENSEN 1942) that the hexose absorption is an active process, made possible through an intermediate, energy-requiring phosphorylation and de-phosphorylation in the intestinal cells) a sugar tolerance test was made in the CO-intoxicated condition. We were prepared to make further experiments in order to analyse the rôle played by the intestine itself and the blood sugar regulating hormones, in case the experiments came out positive. However, our experiments showed no changes in the blood sugar curve during CO-poisoning as compared to a normal curve. Fig. 4 shows two normal blood sugar curves, on E. A. after 100 g glucose in 200 cc of water, on E. Kn. after 70 g in 700 cc water, (the largest dose, that gave no continual rise in the blood sugar curve by this subject) compared to a corresponding curve during CO-poisoning. In neither case the FEHLING test on the urine was positive.

In order to see whether the hemoconcentration occurring during CO-poisoning was due to an increased permeability of the capillaries for water, a series of determinations was made of the filtration out of the vessels of the lower extremity when the hydrostatic pressure was increased by tilting the subject passively to the + 45° position. These experiments showed no increase in the filtration rate after moderate CO-poisoning (in E. A. 9 cc/min. before, 8 cc/min. after CO, in E. Kn. 12 cc/min. before and 8 cc/min. after CO, as the average of 6 pairs of experiments on each subject).

The O<sub>2</sub>-deficiency called forth by the CO-poisoning may be assumed to produce a shift of blood from the central veins and the vessels in the thorax to the periphery. In other cases such a shift of blood has been shown to cause a rise in the vital capacity

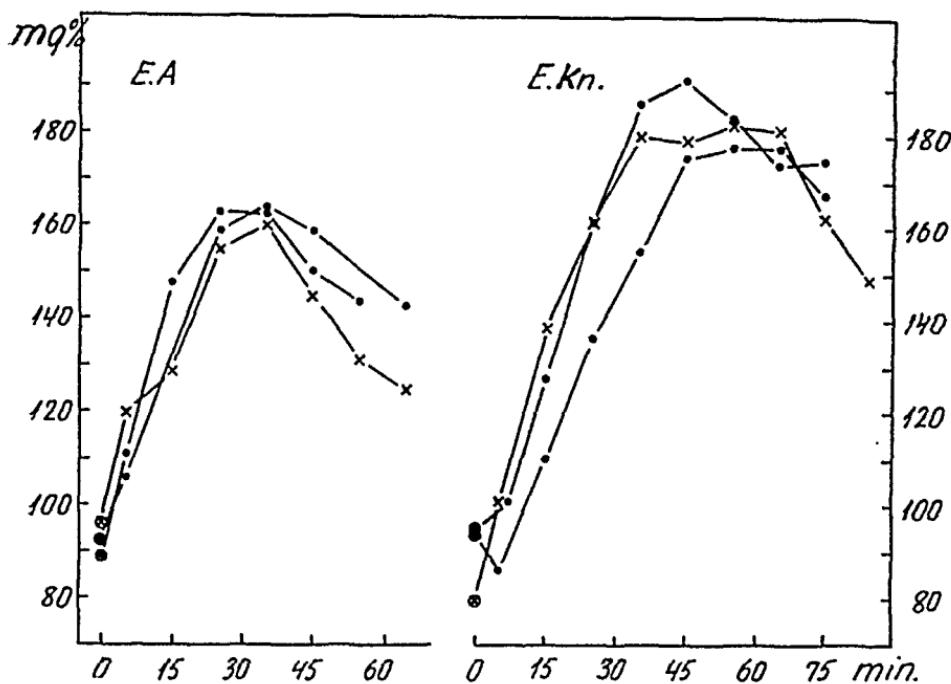


Fig. 4. Glucose tolerance tests on two subjects. ●—● normal condition; ×—× after CO-poisoning. Dose: E. A. 100 g glucose in 200 cc of water. E. Kn. 70 g glucose in 700 cc of water.

(ASMUSSEN, CHRISTENSEN and SJÖSTRAND (1933), SJÖSTRAND (1941) a. o.). We therefore made a series of determinations of the vital capacity before and after a moderate CO-poisoning. The experiments showed, that when the vital capacity was determined at quarter-hourly intervals for one hour before and one after the CO-inhalation, the later determinations gave slightly higher values than the former. However, a series of controls without CO showed the same thing, i. e. the vital capacity rose gradually during the two hours, presumably as an effect of the frequent, maximal expirations. We therefore were not able to observe any shift of blood from the thoracic vessels to the periphery. In passing we may note, that the above mentioned plethysmographic recordings of the leg volume also in this respect fell out negative.

*Discussion.* Our experiments show, that a moderate CO-poisoning, causing a saturation of the blood with CO of between 25 and 32 pCt. of capacity, calls forth a hemoconcentration. The increase in the relative volume of the red cells suggests a compensatory emptying of red cell stores (e. g. the spleen), but the concomitant increase in the concentration of the plasma proteins

shows, that at least a part of the hemoconcentration must be due to a leaking-out of fluid from the blood. Our attempt to demonstrate an increased filtration in the leg during CO-poisoning failed, but it is well possible, that the water is lost, not so much to the muscles or the skin, as to such organs as the liver and the intestine, or it may be excreted through the kidneys. As our results show, the leaking-out of fluid from the blood persists, and in some cases even aggravates at a time, when the CO content of the blood is steadily decreasing. This seems to indicate, that when the balance between blood and tissue fluids is disturbed, primarily by the O<sub>2</sub>-lack, it takes a certain time to get it adjusted again. The large fluctuations in cell volume and plasma protein concentration, which in one case were observed one to two hours after the CO-inhalation, presumably indicate the lability of this balance, when it has first been disturbed.

The reason why fluid is lost from the blood may be sought in an increased capillary blood pressure, caused by a vasodilation, which again is effected through a local O<sub>2</sub>-deficiency. It has formerly been shown (ASMUSSEN and CONSOLAZIO (1941) that a corresponding loss of fluid from the blood stream is the cause of the sudden increase in cell volume and O<sub>2</sub>-binding capacity of the blood observed during the first few days at low O<sub>2</sub>-pressure (high altitude). No doubt, such a hemoconcentration must be kept in mind, when acclimatization to low O<sub>2</sub> or to CO is discussed.

As table 1 and fig. 1 show, the loss of fluid may reduce the total blood volume by from 4 to 8 pCt. It is quite possible that the increased pulse rate always observed during moderate and severe CO-poisoning at least partly is the expression of a compensation for the lowered blood volume. A shift of blood from the central veins to the dilated vessels in the periphery may also, although we were not able to demonstrate it, play an important rôle. The susceptibility to postural changes is increased during CO-poisoning (comp. ASMUSSEN 1942). This, too, may be explained, partly by the diminished blood volume, partly by the dilatation of the peripheral vessels.

For severe CO-poisoning it is generally stated, that there is an increase in the blood lactate, indicating an insufficient O<sub>2</sub>-supply of the tissues. (lit. by BOCK 1923).

In our experiments with moderate HbCO contents of the blood, no increase in the blood lactate was observed during rest. This confirms the results of ASMUSSEN and CHIODI (1941) as well as

those of CHIODI, DILL, CONSOLAZIO and HORVATH (1941), who also found no signs of an increased lactate formation in rest, the later even at HbCO contents above 50 pCt. Quite different are the results from the work experiments: here even a mild CO-poisoning may cause a considerable increase in blood lactate, as seen from fig. 2 as well as from the data of ASMUSSEN and CHIODI (1941). In our present experiments we made careful determinations of the RQ during the steady state of work. It was found that the RQ was higher than normal when CO made the O<sub>2</sub>-supply inadequate. This result, no doubt, has a parallel in the experiments of CHRISTENSEN and O. HANSEN (1939) and of O. HANSEN (1941) who find, that by increasing severity of work, and especially when work is performed with a small group of muscles, (e. g. the arms) the RQ is increased. In unpublished experiments from this laboratory it was found, that such an increase in the RQ was accompanied by an increase in the blood lactate. It is assumed by CHRISTENSEN and O. HANSEN that the increased RQ is a sign of a beginning inadequacy of the O<sub>2</sub>-supply to the working muscles, a conception that is fully confirmed by the present experiments. We found in our two work experiments with CO that the efficiency of the muscles was lowered and the O<sub>2</sub>-debt increased. This may be compared with the similar symptoms in patients with an impaired circulation. A closer evaluation of these results, however, require further experimentation.

Since the days of CL. BERNARD glycosuria has been looked upon as a typical effect of CO-poisoning. Hyperglycæmia has also repeatedly been found following severe CO-poisoning in men (MÜNZER and PALMA, 1894, MOESCHLIN, 1939) and animals (ARAKI, 1891, KELLAWAY 1919, SCHULZE 1936 a. o.). The reason for this has been discussed, and it seems as if both a direct effect on the endocrine glands and an effect produced by the action of the hypoxæmia on the brain are responsible for the hyperglycæmia.

As a CO-poisoning of about 30 pCt. produces a not inconsiderable reduction of the capillary and tissue O<sub>2</sub>-pressure (from 37 mm to 23 mm), it was to be expected that a hyperglycæmia could be detected also in our experiments. Our results, however, showed, that there were neither in rest, nor during work (720 mkg/min.) any signs of a hyperglycæmia. The results of our sugar tolerance tests (fig. 4) further-more showed, that during this grade of CO-intoxication the ability of the liver to remove sugar from the blood was unaffected. Also the kidneys showed no signs of a decreased

ability to hold back sugar from the urine. It must be assumed that the glycosuria and hyperglycæmia observed during CO-poisonings are signs following only *severe* cases of asphyxia.

The general lowering of the O<sub>2</sub>-pressure throughout the organism, which follows even moderate CO-poisonings might be assumed to impair the function of such organs as the intestine. ASMUSSEN, CHRISTENSEN and NIELSEN (1939) have shown, that the ability of the intestine to absorb large quantitatis of water is decreased, when the cardiac output is diminished on assuming the erect posture. Our present experiments showed, (fig. 3), that in one subject (E. Kn.) the same was the case during CO-poisoning. The large quantities of water, that were thrown up during the period of anuria and the almost normal composition of the blood makes it probable that the water actually was not absorbed from the intestine, and that the reason for the low diuresis was not an impairment of the function of the kidneys. As in the above mentioned experiments of ASMUSSEN, CHRISTENSEN and NIELSEN there were no signs of an impaired glucose absorption from the intestine. It must be assumed, that for the absorption of water an abundant flow of blood to the intestinal tract is necessary, and that the CO-poisoning causes a diversion of the blood stream from the intestine to more vital organs.

Our attempts to demonstrate by means of the vital capacity a new distribution of the blood between the thoracic vessels and the periphery in CO-poisoning failed. This of course is not a proof of the non-existence of an altered distribution of the blood during the CO-poisoning. It must be assumed after all that the capillaries of the O<sub>2</sub>-using organs have to dilate in order to supply the necessary amount of O<sub>2</sub>. The increased susceptibility to postural changes is — as mentioned before — a sign of this, and so are the very high pulse rates found in rest and work during CO-poisoning. Finally, the leaking-out of water from the blood, discussed earlier in this paper, is hardly to understand if no such vasodilation occurred, as both arterial blood pressure and cardiac output are practically unchanged by the 30 pCt. CO-poisoning here investigated.

### Summary.

The effect of acute but moderate CO-poisoning (25—32 pCt HbCO in the blood) is studied on two normal men. It is found, that the relative cell volume of the blood as well as the plasma protein concentration are augmented after inhalation of CO,

suggesting a leaking-out of fluid from the vessels, presumably due to peripheral vasodilatations. An increased rate of filtration could not be detected in the lower extremity. The amount of glucose and lactic acid in the blood is unaffected by this degree of CO-intoxication so long as the subject remains quiet. In moderate work the amount of lactic acid increases, whereas the blood sugar still remains normal. The tolerance for glucose is unaffected by this degree of CO-poisoning. In one of the two subjects the intestine lost its ability to absorb large quantities of water, presumably due to a compensatory vasoconstriction in the intestine. An attempt to demonstrate the effect of a shift of blood from the central (thoracic) vessels to the periphery on the vital capacity of the lungs, failed.

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## Experimental Studies on Kidney Function during Sulphate Diuresis.

### 1. Investigations on the Diuresis of Rabbits during Infusion of a Hypertonic Sulphate Solution.

By

PER SCHOU.

Received 14 April 1943.

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According to the modern theory of kidney function, a simple mechanical filtration process takes place in the glomeruli, resulting in an ultrafiltrate of the blood plasma containing the crystalloids of the latter, part of which, during its ensuing passage down the tubules, again passes into the bloodstream. As far as the so-called "threshold substances" are concerned, this takes place at blood plasma concentrations lower than the normal through an *active reabsorption*, while other substances, the so-called "no-threshold substances" — as well as the threshold substances at higher concentrations — are supposed to pass back again through the tubular wall by a simple diffusion process.

According to more recent American investigations a secretion process in the tubules must also be taken into account, by which certain substances pass from the bloodstream into the tubular fluid directly through the tubular cells.

During the study of the above-mentioned facts connected with renal function it was observed that parenteral injection of sulphate has a marked diuretic effect.

Thus already at the beginning of the century MAGNUS (1900), GOTTLIEB (1901), and SOLLMANN (1903) dealt with the diuretic effect of the sulphates.

CUSHNY (1926) thought that this must be due to an increase in

the glomerular filtration in connection with a reduced reabsorption of fluid in the tubules.

More recently CHERRY, EADY and FRAZER (1932) have investigated the diuresis of dogs after ingestion of sulphate on this view, without obtaining conclusive results with respect to the mechanism of the sulphate diuresis.

From our Institute MØLLER (1926) has published some few experiments with infusion of strongly hypertonic sulphate solutions into rabbits, from which excessively large diureses like those in the present experiments were obtained.

In the sequel we shall describe some experiments in which it was possible by intravenous infusion into rabbits of a strongly hypertonic sodium sulphate solution to work up the urine excretion of these animals to about 100—150 times the normal diuresis; the diuresis obtained from the animals, whose normal diuresis ranges around 0.05—0.2 ml. per min., being as high as 16—17 ml. per min.

On considering this excessive diuresis the question naturally arises whether such an excretion of fluid can be explained at all on the filtration-reabsorption theory, the validity of this theory of the renal function being dependent on a positive answer to that question.

It is the object of the present paper and three other papers to follow to try and explain the mechanism of the sulphate diuresis and so to examine the possibility of bringing the observed facts into line with the modern theory of renal function.

*The principle of the investigations* has been to obtain by ingestion of creatinine and analysis of the urine and plasma of the animals with respect to this substance a measure of the degree of concentration of the urine, i. e. a concentration index. This factor multiplied by the diuresis per minute, that is to say, the creatinine clearance, should according to REHBERG give an expression of the ultrafiltrated amount of fluid in the glomeruli, expressed in ml per minute.

The following other factors may now be calculated.

Filtration — diuresis, = the amount of fluid reabsorbed in ml per min.

$$\frac{\text{Diuresis}}{\text{Filtration}} \times 100 = \text{the excretion percentage (Excr. \%)} , \text{ i. e.}$$
  
that part of the filtered amount of the fluid which is excreted with the urine.

100 — Excr. % = Reabs. %, that is to say, that part of the filtered off fluid which according to the calculations must be reabsorbed in the tubules.

### Experimental Method.

The investigations comprise 25 experiments made on rabbits on exactly the same lines. Male rabbits weighing 2 000—4 000 gr. were used. The day before the experiments they had only been fed turnips and water.

All the animals were anaesthetised with urethane 6 ml 25 % solution per kg body weight ingested orally in water, as a rule 50—100 ml, so as to obtain a measurable excretion of fluid for the use of a diuresis period (a "normal period", N), before the sulphate diuresis commenced.

The nature of the experiments with rapid successive diuresis periods required an approximately accurate measure of the diuresis over quite short periods and a corresponding quick taking of blood samples. A bladder cannula was therefore inserted in the vesica as far up as the entry of the ureters.

The infusion took place through the exposed jugular vein from a 100 ml burette devised like a Wolff's flask. By a suitable warming device the temperature of the infusion fluid could be regulated in accord with the body temperature of the animal.

Blood samples were drawn in the middle of the diuresis periods through a cannula inserted in the carotid artery. Coagulation in the cannula was prevented by citrate, in the blood samples by means of Liquoid Roche. The blood was at once centrifuged, whereupon the analyses were made on plasma.

The creatinine analyses on the plasma and urine were performed on the principle of FOLIN, with a technique devised by NIELSEN (1933).

### Experimental Results.

For the description of the sulphate diuresis experiments we have chosen a single experiment typical of all 25, which were carried out with essentially the same technique.

#### *Notes on experiment:*

Experiment 18,  $\frac{1}{2}$  1940. Male rabbit. Weight: 3.26 kg. Temperature at beginning of experiment  $37.8^\circ$ ; at end of experiment  $39.2^\circ$ .

At 9.30 oral ingestion of 1.5 gr. creatinine and 19.5 ml 25 % urethane solution in up to 100 ml water. Animal placed in an incubator.

At 10.30 operation as described above: bladder cannula, cannula in left carotid artery for blood samples; further, a cannula in the right jugular for infusion.

12.35—13.55 urine collected and measured. Diuresis 3.8 ml in 80 min. =  
   = 0.048 ml per min.  
 At 13.05 blood sample drawn, 6 ml<sup>1</sup> for clearance determination in a  
   diuresis period ("normal period") before sulphate diuresis  
   sets in.  
 14.05—14.27 intravenous infusion first of 20 ml 0.9 % NaCl solution  
   and then of 125 ml 20 % Na<sub>2</sub>SO<sub>4</sub>, 10H<sub>2</sub>O solution, i. e.  
   at an infusion rate of 6—7 ml per minute.  
 14.15—14.19 First sulphate diuresis period ... Blood sample (6 ml)  
   at 14.17. Diuresis 45 ml in 4 min. 11.25 ml per min.  
 14.19—14.23. Second sulphate diuresis period ... Blood sample  
   (6 ml) at 14.21. Diuresis 65 ml in 4 min. = 16.25 ml per  
   min.  
 14.23—14.27. Third sulphate diuresis period ... Blood sample (6 ml)  
   at 14.25. Diuresis 32 ml in 4 min. = 8.00 ml per  
   min.

Experiment concluded, animal killed, kidneys removed and fixed  
 (bodywarm) for histological examination.

The results of this and 5 similar cases chosen at random are  
 presented in Table 1.

The corresponding conditions for all 25 experiments are shown  
 in Fig. 1, where each point in the curve represents a single exper-  
 imental period after sulphate infusion, the normal periods being  
 omitted here.

The experiments show the following facts (compare Table 1  
 and Fig. 1).

1) *The diuresis is enormously increased as a result of the sulphate infusion, e. g. in the above-described experiment 18 from 0.05 ml per min. in the normal period to respectively 11.25, 16.25, and 8.00 ml per min. in the 3 four-minute sulphate diuresis periods.*

In most of the other experiments the same considerable in-  
 crease was found in the diuresis. The great rise in the excretion  
 of fluid sets in as soon as the sulphate has reached the animal's  
 blood-stream, and on continued sulphate infusion attains its  
 maximum in about 10—12 minutes, whereafter there is again a  
 decrease in the excretion of urine so that after the infusion has  
 lasted for about 20 minutes (the duration of the infusion in most  
 of the experiments) the excretion has dropped to about 1 ml  
 per min., i. e. still a high diuresis for a rabbit.

<sup>1</sup> The reason why the blood samples were so comparatively large was that in these experiments determinations were also made in the various periods of the serum protein content and the haemoglobin percentage, while the plasma and urine were analysed for various substances; sulphate, urea, chloride, and glucose. The results of these investigations will be discussed in a later paper.

Table 1 shows the observed and calculated factors relating to the movement of the fluid from 6 typical sulphate diuresis experiments (Experiments 15—20 incl.).

For each experiment the table includes a pre-period (N) before the sulphate infusion and 3 sulphate diuresis periods ( $S_1$ ,  $S_2$ , and  $S_3$ ).

Table 1.

Experiment	Period	Creatinine-		Urine-C. index	Creatinine- clearance ml per min.	Diuresis ml per min.	Reabs. fluid ml per min.	Excr. %	Reabs. %
		serum mg %	urine mg %						
15	N	17.10	2130	125	11.85	0.095	11.76	0.8	99.2
	$S^1$	11.03	22.3	2.02	26.30	13.00	13.30	49	51
	$S^2$	10.50	20.3	1.93	24.00	12.40	11.60	52	48
	$S^3$	11.95	22.8	1.91	16.25	8.50	7.75	52	48
16	N	32.6	1764	54.1	7.57	0.14	7.43	1.9	98.1
	$S^1$	25.0	52.2	2.10	16.25	7.75	9.50	48	52
	$S^1$	24.0	34.9	1.45	17.22	11.88	5.34	69	31
	$S^3$	25.9	36.5	1.41	10.91	7.75	3.16	71	29
17	N	24.6	3390	138	8.96	0.065	8.90	0.7	99.3
	$S^1$	20.0	84.5	4.22	34.80	8.25	26.55	24	76
	$S^2$	20.2	38.7	1.92	29.50	15.38	14.12	52	48
	$S^3$	20.6	30.1	1.46	18.10	12.38	5.72	68	32
18	N	16.0	4000	250	12.00	0.048	11.95	0.4	99.6
	$S^1$	10.8	21.6	2.00	22.50	11.25	11.25	50	50
	$S^2$	10.2	15.4	1.51	24.50	16.25	8.25	66	34
	$S^3$	10.0	19.8	1.98	15.85	8.00	7.85	50	50
19	N	14.7	4450	303	13.60	0.045	13.16	0.3	99.7
	$S^1$	12.4	35.7	2.88	21.60	7.50	14.10	35	65
	$S^2$	11.5	17.8	1.55	23.60	15.25	8.35	65	35
	$S^3$	11.7	17.8	1.52	16.70	11.00	5.70	66	34
20	N	44.7	6150	138	10.80	0.078	10.72	0.7	99.3
	$S^1$	39.7	70.2	1.77	20.60	11.63	8.97	56	44
	$S^2$	34.7	49.7	1.43	22.20	15.50	6.70	70	30
	$S^3$	33.8	57.5	1.70	14.00	8.25	5.75	59	41

Fig. 1 shows that the diuresis differed in the various periods. The highest values for the excretion of fluid, 15—17 ml per min., were attained for the largest of the animals and most frequently in the second sulphate diuresis period, while the comparatively lower values, down to less than 1 ml per min., were chiefly derived from the last periods of the experiments and especially from a few experiments where the further course of the falling diuresis

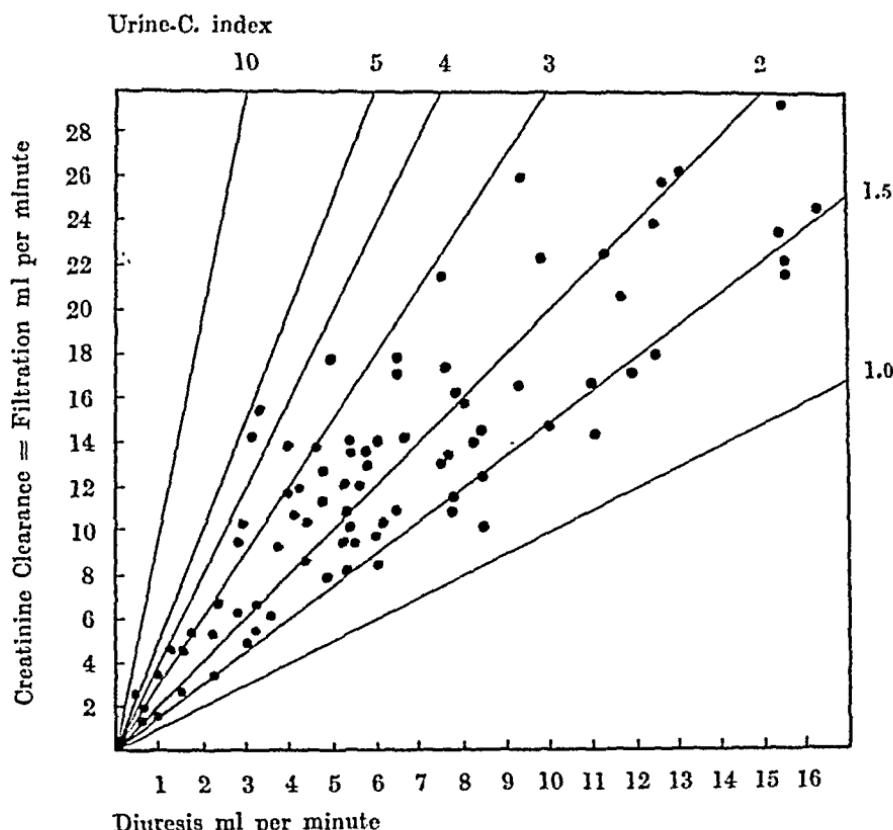


Fig. 1. Diagram showing the relation between diuresis and filtration in the glomeruli calculated as the creatinine clearance in 84 sulphate diuresis periods in 25 experiments.

The points represent the relation found in each diuresis period.

The oblique lines represent some values chosen at random for the C index of the urine determined according to the definition  $\frac{\text{ultrafiltration}}{\text{diuresis}} = \text{C. index}$ .

curve was followed beyond the diuresis periods analysed in the "standard experiments".

These experiments will be discussed in a later paper.

2) *The creatinine clearance*, which in accordance with the prevailing renal function theory should in rabbits be a measure of the ultrafiltration in the glomeruli, increases at the same time though in less degree than the diuresis. Within the sulphate diuresis periods (Fig. 1) the creatinine clearance seems to increase in direct ratio with the increase in the diuresis.

3) *The concentration degree of the glomerular filtrate, i. e. the concentration index of the urine (C. index)* drops from values about 100—300 (Table 1) prior to the sulphate infusion to about 1.5—2.0—4.5 after sulphate has been given.

## Histological Examination of the Kidneys after Sulphate Diuresis.

In several of the experiments the kidneys were removed for histological examination. Similar examinations have previously been made by Danish investigators.

Thus SCHEEL (1907) showed on rabbits' kidneys that the infusion of a sulphate solution produces a considerable dilatation of the tubules.

JESSEN (1923) confirmed this fact.

OKKELS (1930) in his doctor's dissertation described the histological picture of a rabbit's kidney after infusion of a strongly hypertonic sulphate solution.

The author found a varied picture of the tissue, much dilated capillary loops in the glomeruli and slight dilatation of the glomerular capsule. In the tubules, whose different segments could each be distinctly discerned, all transitions from the finest slitlike to more dilated lumina with cupola formation were found. In certain places Henle's loops were found to be full of these cell cupolas from the first convoluted tubule, evidently carried distally with the stream.

In OKKEL's experiment the animal had had an infusion of 6 ml 20 %  $\text{Na}_2\text{SO}_4$ . In the experiments here described the animals had been given an infusion of considerably larger amounts of sulphate solution and in the histological picture of these experiments the above-mentioned changes do in fact recur but in a more marked degree than has previously been observed.

As an example we may here describe the kidney from the above-mentioned Experiment 18.

*Macroscopically* the kidney presented nothing special and the capsule was easily detachable.

*Microscopically*<sup>1</sup> the typical feature in the picture of the kidney is a pronounced dilatation of all channels.

BOWMAN's capsules give the general impression of being enlarged, more or less as it were pressing together the adjacent tubules. The capsular leaf of the glomerular epithelium is distinctly flatter than normal, so that the distance between the separate cell nuclei is remarkably large.

The capsular space itself is large presumably as a result of a dilatation of the capsule.

In the glomeruli there is a distinct dilatation of the capillaries with comparatively few blood cells and very slightly stained plasma.

Some of the connective tissue cells in the supporting tissue of the glomeruli give the impression of being edematous, and altogether the glomerulus is light-coloured.

As regards the tubules it is primarily the pronounced dilatation of the lumina that dominates the picture, while in the cortical zone it is

<sup>1</sup> I am much indebted to Prosector L. HEEREF for helpful advice in the description of the histological preparations.

impossible to distinguish between the proximal and the distal tubular loops.

The cells in the convoluted tubules are lower than normal but otherwise present no distinct signs of degeneration. The nuclei are well preserved with visible cell membranes. The protoplasm is granular or radiately striated and in some few places remains of brush seams are seen. However, the cells have in many places a peculiar irregular appearance towards the lumen partly perhaps originating from precipitations in the lumen which extend into the protoplasm. No cell limits are to be seen.

In *Henle's thin segment* the lumen is enormously dilated with quite flattened cells with strongly projecting nuclei.

The cells of the *collecting tubes* are light-coloured, "vesicular", and here again the cells, as throughout the kidneys, are enormously dilated.

The connective tissue stroma has also, around the tubules, an edematous appearance, the capillaries are dilated, but otherwise there is no change in the vessels.

Thus the histological picture exclusively shows evidence both in the glomeruli and especially in the tubules of the enormous amount of fluid that passes through the renal ducts in a short time.

### Discussion.

As far as we know there have not previously appeared in the literature any more detailed investigations on so excessive an increase in the diuresis of rabbits as was produced in the experiments here described.

If the relation between the *diuresis* and the *creatinine clearance* is examined, Fig. 1 and Table 1 show that corresponding to the very substantial increase in the excretion of fluid there occurs an increase in the creatinine clearance which — within a very considerable range of variation — rises in the sulphate diuresis periods to about twice or three times the values observed in the normal periods. The position of the points in Fig. 1 would seem to indicate a distinct tendency to direct proportionality between the two factors, and even if the diuresis is augmented ever so much there is nothing to show that the creatinine clearance adjusts itself to any maximal constant value ("augmentation limit").

There are pretty considerable divergences in the diagram but in this connection it should be noted that the results presented here are derived from 25 experiments.

The straight line around which the points are grouped in fig. 1 represents a remarkably low *C. index* of about 2, varying between 1.3 and about 4; these extremely low values for *C. indices*, presumably not previously observed in intact animals, in connection with the very high diuresis must be pointed out as characteristic of the sulphate diuresis.

In man (COPE 1932, IVERSEN and BJERING 1935) and in certain animals: anthropoid ape, bird, and dogfish (quoted from SMITH 1937), the creatinine clearance, according to the modern kidney function theory, is assumed not to be identical with the filtration in the glomeruli, creatinine being excreted partly by tubular excretion (SHANNON 1935), so that in these animal species as recommended by SMITH (1937) inulin clearance must be used as a measure for the glomerular filtration.

In rabbits (KAPLAN and SMITH 1935), dog, sheep, and seal (quoted from SMITH 1937), on the other hand, creatinine clearance is said to give a quantitative measure of the amount of the filtration.

If we adapt this view to the experimental results gained in the present work the excessive sulphate diuresis must be supposed to come about as follows:

*The ultrafiltration is augmented 2—3 times. The essential factor in the increase of the diuresis, which amounts to about 100—150 times, must therefore be a reduction of the reabsorption in the tubules, or in other words: the diuresis is partly conditioned by the glomeruli, but quite predominantly by the tubules.*

These facts are presented in the last two columns of Table 1, which show the values for the excr. percentage and the reabs. percentage calculated in accordance with the filtration — reabsorption theory, i. e. the percentage figures for those parts of the glomerular filtrate which are respectively excreted and reabsorbed in the tubules.

While, as will appear from this, under normal circumstances less than 1 % of the glomerulus filtrate is excreted as urine, the remaining 99 percent being reabsorbed in the tubules, these conditions must be altered as a consequence of the sulphate infusion in such a way that hereafter about 50—70 % of the amount of fluid filtered in the glomeruli will be excreted and the reabsorbed amount of fluid thus be reduced correspondingly so as to comprise only 50—30 %, a *C. index* of 2 signifying that 50 % of the filtered off fluid are reabsorbed while 50 % are excreted with the urine. *C. index* 3 = 67 reabsorption, 35 % excretion.

This result can in no way be said to be irreconcilable with the kidney function theory but, on the other hand, the experimental results cannot be taken as a proof of the validity of the theory.

Thus certain presuppositions must be made if the sulphate diuresis is to be explained on the filtration-reabsorption theory.

As regards the *glomerulus function* physical conditions allowing the calculated augmentation of the filtration must be present in the glomeruli.

In another paper it will be shown that the conditions of blood-pressure and blood-flow obtaining at any time in the glomeruli are of such an order of magnitude that the range of variation found for the filtration — from less than 1.0 to 30.0 ml per min. — becomes possible (fig. 1).

With respect to the *tubular function* it should be noted that, assuming the correctness of the theory, the urine should, during the excessive sulphate diuresis, come very close in its composition to the composition of the ultrafiltrate when as much as 60—70 % of this is excreted as urine. In a future paper, where the content in the urine of various substances is determined and compared with the corresponding concentrations in the plasma and the concentrations in the ultrafiltrate calculated from these, it will be shown that during the excessive sulphate diuresis the urine actually approximates very closely to the glomerular ultrafiltrate.

In this connection we may mention a work by BAYLISS and LUNDSGAARD (1937). By perfusion experiments on isolated kidneys with blood containing cyanide these authors, as previously STARLING and VERNEY (1924), brought about a greatly increased diuresis. This was supposed to result from a paralysis of the reabsorption in the tubules caused by the cyanide, expressed by a fall in the C. index to the same level as was obtained in the present work by the infusion of sulphate.

A similar reduction in the concentration index of the urine in perfusion experiments on isolated kidneys was found by BICKFORD and WINTON (1937) by cooling.

In both the above-mentioned works it proved impossible to stop the tubular activity of the kidneys entirely, nor has this been possible in the present investigation. The C. index is not in any case pressed down to 1.0, even though the lowest values are indeed not far from it (1.2).

Incidentally, as regards the glomerulus function it is interesting to note that, while in the present work an increase in the filtration must, as already mentioned, be taken into account as one factor in the augmentation of the diuresis, the calculations in the works of the above-mentioned authors on the contrary show a fall in the filtration, which is presumably due to the fact that they deal with an artificial perfusion of the kidneys and so with partly injured kidneys.

The *histological picture* of the kidneys from the animals used for the experiment shows changes with dilatation of the glomeruli but especially — and in a marked degree — of the ducts of the kidneys both the convoluted tubules, the loops of Henle, and the collecting tubes — a picture which will readily explain how the enormous amounts of fluid have been able to pass through the kidneys in as short a time as was the case.

### Summary.

1) By intravenous infusion of large amounts of hypertonic sulphate solution the diuresis of rabbits is brought up from the normal order of magnitude of about 0.1 ml per min. to values ranging from 10—15 ml excreted fluid per minute, that is to say, an augmentation to about 100—150 times the normal diuresis.

The creatinine clearance is increased to about 2—3 times the normal, while the figures for the C. index of the urine drop from 100—200 to about 2.

2) If the creatinine clearance is accepted as a measure for the ultrafiltration in the glomeruli, calculations of the movement of the fluid in the kidneys in accordance with the filtration — reabsorption theory show that the enormous diuresis must occur as an augmentation of the glomerular filtration combined with a very marked reduction of the reabsorption of the fluid in the tubules, half to two-thirds of the glomerular filtrate leaving the kidneys as urine under these circumstances.

3) The histological picture of the kidney after sulphate diuresis shows an enormous dilatation of all the renal ducts, which explains how the immense amounts of fluid have been able to pass through these in the course of a very short time.

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## Studies on the Muscular Functions of the Genital Tract.

### IV. Tonus of the Uterine Musculature in the Rabbit; its Dependence on Hormonic Factors.

By

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Received 3 May 1943.

Earlier investigations (GENELL, 1937, 1939, 1940) have shown that the tonus of the uterine musculature in the rat depends upon hormonic factors. Oestrin lowers the tonus, and as a result the amplitude increases and the contraction frequency decreases in the uterine musculature. This effect of oestrin subserves, among other things, the mechanism of sperm transport (GENELL, 1939), which in the rat, as in other animals in which it has been possible to investigate it experimentally, takes place with great rapidity. In most of the animals studied the spermatozoa are transported from the vagina to the tube within one minute. At the time of my first investigation (GENELL, 1937) the rabbit alone seemed to occupy a distinct position, the sperm transport in this animal appearing to require one to two hours. In view of this it would have been expected that the basic fact in this mechanism, the depression of tonus by oestrin, did not apply to the rabbit. Since then, however, a new investigation (KREHBIEL and CARSTENS, 1939) has made it probable that the mechanism of sperm transport in the rabbit is fully analogous to that in other animals and takes place with the same extraordinary rapidity. In these circumstances it became desirable to investigate the tonus conditions in the uterine muscle of the rabbit and the effect upon them of oestrin.

*Method.* The same method has been applied as in the investigation on the tonus conditions in the rat's uterine muscle (GENELL, 1940). On account of the size of the organ in the rabbit, however, only a 2 mm broad ring from the middle of one uterine cornu has been taken instead of the whole cornu. The recording threads have been looped loosely through this ring, and it is therefore the tonus of the circular muscle that is recorded.

In measuring the relaxation curves the peak of the first contraction has not been selected as the zero position, as it was in the aforementioned experiments, but the average of all the contraction peaks has been taken as the zero position. As it is known from previous experiments on rats that the tonus is not stabilized in the surviving preparation until after about one hour, only the relaxation values from 60 to 180 minutes have been recorded.

Since the sexual cycle of the rabbit, on account of this animal's coitus-controlled ovulation, is irregular and difficult to determine, the experiment has been conducted on animals castrated one month before the experiment. At the first operation, in which the first uterine cornu was extirpated for trial, 10,000 I. U. of oestrin (in oil solution) were given intraperitoneally, and the second cornu, thus treated, was examined six days later. Twenty rabbits have been employed.

The result of the investigation is given in Table 1, in which the relaxation values are recorded. It is obvious that the difference between the groups "Castrate" and "Oestrin-treated Castrate" is quite significant.

*Discussion.* The present investigation shows that the effect of oestrin on the tonus of the uterine musculature of a castrated animal is the same in the rabbit as in the rat. The tonus is depressed by the action of oestrin. It is, then, justifiable to draw the conclusion that the variations in the tonus of the uterine musculature detected in the rat during different phases of the sexual cycle are likewise present in the rabbit. The heat period is, then, characterized by low tonus in the uterine muscle, the other phases by higher tonus, the higher the lower the oestrin elimination is.

*Summary.* The tonus of the myometrium of the rabbit is high in castrates. It is depressed by pretreatment of the experimental animal with oestrin. By analogy the conclusion is drawn that the tonus of the uterine musculature in the rabbit varies with the sexual phases in the same way as in the rat, that it is low during the heat phase, high during the non-heat phase.

Table 1.

Animal	60'	70'	80'	90'	100'	110'	120'	130'	140'	150'	160'	170'	180'
Castrates	Rabbit 1	0.0	0.0	0.5	1.0	1.0	1.0	1.5	1.5	2.0	2.5	2.5	3.0
	2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	1.0
	4	2.0	1.0	0.5	0.5	1.0	0.5	0.5	0.5	1.0	1.0	1.0	1.5
	5	7.5	7.5	7.5	7.5	9.0	10.5	10.0	11.0	12.0	13.0	14.0	13.5
	6	3.5	1.5	0.5	0.5	1.0	1.0	1.0	1.5	2.0	2.5	2.5	3.0
	7	1.5	1.5	1.5	2.0	2.0	2.0	2.5	3.0	3.0	3.0	3.5	4.5
	8	4.5	0.5	0.5	0.5	0.5	0.0	0.5	0.5	1.5	1.5	2.0	3.0
	9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
	10	5.0	0.0	0.0	0.0	0.5	0.5	0.5	0.5	1.0	1.0	1.5	2.0
	11	5.0	3.5	3.0	3.5	4.0	4.0	4.5	4.5	5.0	5.5	6.0	6.0
	12	1.0	1.5	1.0	0.0	0.0	0.0	1.5	2.0	2.5	2.5	3.0	4.0
	13	1.0	1.0	1.5	1.5	1.5	1.5	2.0	2.0	2.5	2.5	2.5	3.0
	14	1.0	1.0	0.5	1.5	2.0	2.5	2.5	3.5	4.0	4.5	4.5	5.0
	15	1.0	1.0	0.5	1.0	1.0	1.0	1.0	1.5	2.0	2.0	2.5	3.5
	16	5.0	5.5	5.0	5.5	5.5	6.0	6.0	5.5	6.0	6.0	6.0	6.5
	17	1.5	1.0	0.5	1.0	0.5	1.0	1.0	1.5	2.0	3.0	3.5	3.5
	18	0.5	0.0	0.0	0.5	1.0	1.0	1.0	1.5	1.5	1.5	2.0	2.5
	19	2.0	1.5	2.0	2.0	2.0	2.0	2.5	2.5	3.0	3.0	3.5	4.0
	20	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.5	0.5	1.0	1.0	1.5
Oestrin-treated castrates	Mean	2.1	1.4	1.2	1.4	1.6	1.7	1.9	2.1	2.5	2.7	3.1	3.5
		$\pm 0.5$					$\pm 0.6$						$\pm 0.7$
Oestrin-treated castrates	Rabbit 1	7.0	8.5	9.5	11.5	12.5	13.0	13.5	13.5	13.5	13.5	14.0	14.0
	2	4.5	4.0	4.0	5.5	6.5	7.0	8.0	8.5	9.0	9.5	9.5	10.0
	3	4.5	6.0	6.5	7.0	7.5	7.5	7.5	8.0	7.5	7.5	7.5	8.0
	4	6.5	6.0	6.0	6.0	6.0	6.0	6.0	6.5	7.0	7.0	7.0	7.5
	5	10.0	9.5	10.0	10.5	12.0	12.0	12.5	12.5	13.0	13.0	12.0	12.0
	6	5.5	5.0	6.0	7.0	7.5	7.5	8.0	8.5	9.0	9.0	9.0	9.0
	7	3.5	3.0	4.5	5.5	6.5	6.5	7.0	7.5	8.0	8.5	8.5	9.0
	8	9.0	10.0	10.5	11.0	13.0	13.5	14.0	13.5	14.0	15.0	15.0	15.5
	9	3.0	3.0	3.5	4.0	4.5	4.5	5.0	5.5	5.5	5.5	6.0	6.5
	10	6.0	5.5	6.0	5.0	5.5	6.0	5.5	5.5	5.5	5.5	5.5	6.0
	11	5.5	5.0	5.5	5.5	4.5	5.0	4.5	4.5	5.5	5.5	5.5	6.0
	12	9.0	11.0	10.5	10.0	10.5	12.0	13.0	13.5	14.0	14.5	14.5	15.0
	13	11.5	13.0	13.5	14.0	14.5	14.5	14.5	15.0	15.0	15.0	15.5	15.5
	14	0.0	0.5	1.5	2.0	2.5	3.5	4.5	4.5	4.5	5.0	5.0	5.0
	15	10.0	10.0	10.5	11.5	12.5	13.0	13.0	13.5	14.0	14.0	14.5	14.5
	16	7.0	8.0	9.0	8.5	9.0	11.5	12.0	13.0	13.0	13.5	13.5	14.0
	17	5.5	6.0	7.0	7.0	8.0	9.5	10.0	11.0	11.0	11.5	11.5	12.0
	18	4.5	4.0	4.5	5.0	5.5	6.0	7.5	8.0	8.5	9.0	9.5	10.0
	19	10.0	11.0	11.0	11.0	12.0	12.5	13.0	13.0	13.0	12.5	12.5	12.0
	20	6.5	6.5	7.0	7.0	6.5	6.5	6.0	6.0	7.0	7.0	7.5	7.5
Oestrin-treated castrates	Mean	6.4	6.7	7.3	7.7	8.3	8.8	9.2	9.5	9.8	10.1	10.1	10.5
		$\pm 0.6$					$\pm 0.9$						$\pm 0.8$

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## On the Influence of Alcohol on the Pupillary Light Reflex in Man.

By

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Received 17 May 1943.

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A critical perusal of the protocols of the tests carried out by different physicians on persons under the influence of alcohol showed contradictory observations on the presence of slowed pupil reactions (LILJESTRAND 1940). A study of this reaction with an objective method was therefore deemed necessary in order to elucidate the diagnostic value of this symptom.

To this end the pupil contraction was recorded with a 16 mm film camera at a controlled rate, generally 20 pictures per sec. The diameter of the pupil was measured directly from the film in a microscope with an ocular micrometer permitting an accuracy of 0.1 mm. The observer gazed monocularly against a faintly illuminated ground glass disk of about 5 cm in diameter at a distance of 30 cm. The rest of the room was dark. The ground glass disk could be instantaneously illuminated with a 200 watt bulb in a projector with the aid of a shutter. 15 ml 50% alcohol was given on an empty stomach. In all, six experiments were carried out with four observers. Blood alcohol was measured in three cases.

As shown by fig. 1, the normal reflex contraction of the pupil took place along a logarithmic curve the constant of which varied with the initial width of the pupil. This fact has previously been noted by MACHEMER (1935). C represents a curve with a medium initial pupil size, F one after maximal dilatation obtained by prolonged dark adaptation, A and B after smaller initial values

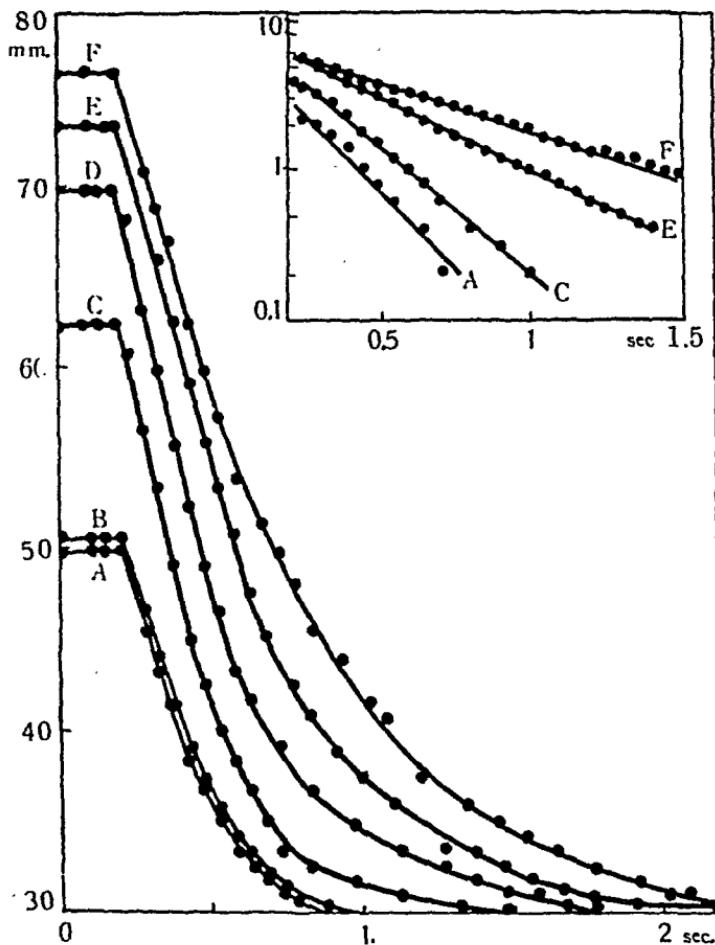


Fig. 1. Contraction curves for pupil reacting to light. Ordinates: pupillary diameters in mm. Abscissae: time from beginning of stimulus in sec. Inset: Ordinates: log pupillary diameter less the diameter of the pupil when maximally contracted.

caused by respectively stronger fixation light and pilocarpine in the conjunctival sack. Subjective tests are directed towards observation of the rapid initial phase. For ordinary pupil widths the initial phases differ too little to be noticeable to the naked eye.

Alcohol dilates the pupil in proportion to the blood alcohol by roughly about 10 % for maximum concentration of alcohol in these experiments, 1.3 promille. The pupillary contraction curves from such experiments as in D and E in fig. 1, corresponding to a blood alcohol of about 0.5 respectively 1.2 promille, do not differ from the normal ones, but as with large pupillary diameters

it lasts somewhat longer before the contraction is finished, this may simulate a slowening of the pupil reflex.

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## The Gastric Secretory Excitant from the Pyloric Mucosa.

By

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Received 6 May 1943.

EDKINS (1906) reported that extracts from the pyloric mucosa of pigs and cats evoked gastric secretion when injected intravenously on anaesthetized cats. The most active extracts were obtained by boiling the mucosa in water or 0.4 % HCl. Extracts from the fundic mucosa were inactive, whereas cardiac mucosal extracts showed slight activity. From these experiments EDKINS postulated the existence of a humoral mechanism acting in the gastric phase of gastric secretion. Secretory excitants were considered to be liberated chiefly from the pyloric mucosa.

Later experimental work has convincingly proved that a humoral mechanism controls the chemical phases of gastric secretion. The results obtained have also provided evidence in favour of the view that the humoral factor is chiefly liberated from the pyloric mucosa. The *hormonal* nature of the agent, however, has been doubted.

A review of the numerous papers concerning these problems was given by Uvnäs (1942). In the present work only a few investigations dealing with the chemical nature of the humoral factor will be cited.

MAYDELL (1913) arrived at the same conclusions as EDKINS. In his experiments HCl-extracts from the gastric mucosa of pig, dog and cat were tested on Pavlov pouch dogs. Working on anaesthetized cats, LIM (1923), also observed a gastric secretory excitant in HCl-extracts from the pyloric mucosa, but as he failed

to find any excitant in the blood after meals he was of the opinion "that EDKINS's gastrin must be regarded as an extraction produkt rather than as an internal secretion product". Other investigators (POPIELSKI 1909, EMMANN 1912, EHRENMANN 1912) found that HCl-extracts from various mucous regions of the alimentary canal evoked gastric secretion on subcutaneous or intravenous administration. KEETON and KOCH (1915) demonstrated the occurrence of a gastric secretory excitant in extracts from all regions of the dog's gastric mucosa. As extracts from other regions of the alimentary canal were inactive, they considered the active agent to be a specific gastric excitant. The active agent was obtained in a protein fraction insoluble in absolute alcohol. In a later paper LUCKHARDT, KEETON, KOCH and LA MER (1919) report that the alcohol-soluble fraction of their extracts also showed activity. An alcohol-soluble substance could be extracted from various mucosal regions of the alimentary canal, as also from several other tissues and organs. The nature of these "gastrin bodies" was further investigated by KOCH, LUCKHARDT and KEETON (1920). They considered them to be closely related to but not identical with histamine.

POPIELSKI (1920) observed the gastric secretory effect of histamine. When SACHS, IVY, VANDOLAH and BURGESS (1932) were able to isolate crystalline histamine from extracts of the pig's pyloric mucosa, this was taken to indicate that histamine was "the gastric hormone". The secretory activity of EDKINS' extracts, as also those of later investigators now was considered to be due to their histamine content.

In 1938 KOMAROV claimed to have obtained from the pyloric mucosa of dogs a histamine-free extract, which on intravenous injection on anaesthetized dogs and cats evoked gastric secretion. Fundic extracts were inactive, whereas extracts from the duodenal mucosa showed slight activity. The active agent was found in the protein fraction and considered to be protein like in nature. KOMAROV considered his findings to support the theory of a hormonal mechanism acting in the gastric and intestinal phases of the gastric secretion. The results of KOMAROV, however, could not be confirmed in IVY's laboratory (IVY 1940).

In 1942 UVNÄS reported experiments which indicated that the cephalic phase of gastric secretion is controlled by a hormonal mechanism chiefly localized in the pyloric region. Interference with the function of the pyloric mucosa by cocainization or extir-

pation stopped or considerably diminished the gastric secretion induced by electrical stimulation of the vagi. In cross-circulation experiments a gastric secretory excitant was observed in the blood during vagal stimulation. By using KOMAROV's extraction technique, extracts from the dog's, cat's and pig's pyloric mucosa were found to stimulate gastric secretion. Fundic extracts were inactive. UVNÄS agrees with KOMAROV that the active agent is not histamine, but probably a protein-like substance.

### Experimental Technique.

All experiments were performed on cats and dogs previously starved for 24 hours. The anaesthetic used was a mixture of chloralose and urethane. During a short ether anaesthesia 0.05 gm chloralose and 0.5 gm urethane per kg body weight were slowly injected intravenously. Before anaesthesia the dogs received 10 mg morphine per kg body weight subcutaneously.

The operative procedure was identical with that devised by UVNÄS (1942). The vagi were exposed in the neck and cut. The trachea was cannulated and the oesophagus tied in the neck. The stomach was drained by a thin perforated rubber tube introduced into the stomach through a glass cannula inserted in the ventral stomach wall just proximal to the pyloric region. The cannula with the rubber tube was pushed out through an incision in the left abdomen. The duodenum was ligated just proximal to Vater's ampulla. After the abdominal wall was closed the animal was placed on its left side, the front half of the body somewhat higher to facilitate the outflow of the gastric juice.

Our earlier extracts (UVNÄS 1942) were investigated on animals with their pyloric regions cocainized or extirpated. It was then observed that a maximal secretory response was usually obtained only when the vagi were electrically stimulated concomitantly with the injection of extracts. In our later experiments the extracts have been tested on animals with their stomachs intact. On these animals abundant secretion was induced by the extracts even without concomitant vagal stimulation. To ascertain a maximal secretory response, however, in most experiments the vagi were stimulated by the technique previously used. The intensity of the stimulus was adapted to maintain only a very slight continuous gastric secretion. The secretory volume was measured in 15-minute periods. The gastric juice was tested for total acidity either by electrometrical titration a potentiometer according to Prahn being used (UVNÄS 1942), or colorimetrically using phenolphthalein as indicator and N/10 NaOH as base.

## The Secretory Response to Extracts from Different Mucosal Regions of the Stomach and Intestine of Cats, Dogs and Pigs.

The extraction technique devised by KOMAROV (1938) was used. During the course of the work minor modifications were made. Most of the extracts were prepared in the following way.

The mucosa to be extracted was excised, minced with scissors and boiled for 30 minutes in 10 volumes of N/10 HCl. The acid extract was then left in the refrigerator over night. Next morning after centrifuging and filtering through cotton wool the filtrate was neutralised with N NaOH to pH 4—5 and precipitated with an equal volume of 10 % trichloracetic acid. 1—2 hours later the precipitate was centrifuged off and washed 3 times with 40—50 volumes of 10 % trichloracetic acid in saline, twice with 50 volumes of acetone, once with benzene, twice with ether and dried at 37° C. A white powder was obtained, which was then stored in the refrigerator.

Before testing, the extracts were suspended in Tyrode solution made slightly acid with a few drops of N/10 HCl. An opalescent solution was obtained. In testing extracts from dogs and cats an amount of dry substance corresponding to 2—5 gm of moist mucosa was suspended in 15 ml of Tyrode solution. In testing extracts from pigs amounts corresponding to 5—10 gm mucosa were used.

The testing procedure was as follows. The vagi were slightly stimulated to induce a slow constant gastric secretion (some tenths of ml per 15 minutes). Constancy of secretion being obtained, the extract was injected intravenously in the iliac vein at a rate of 0.4 ml per minute. After 30 minutes the injection was stopped and the secretion allowed to decline to the basal level before a new extract was tested.

*Fig. 1 a* shows a typical secretory response to the injection of a pyloric extract from cat and *fig. 1 b* the response to two extracts from pig's pyloric and one from pig's fundic mucosa. The secretion usually began to increase 5—10 minutes after the injection had started, and reached its maximal value during the second 15-minute period. After the injection was stopped an abundant secretion continued during the following 15 minutes, and then gradually declined, usually to reach the basal level within 30—45 minutes after the end of injection. The acidity of the juice varied in the different experiments, but was always high. As in the secretion induced by vagal stimulation or histamine injection, the acidity gradually rose during the first period of secretion, later to be maintained at a relatively constant level. The acidity usually rose to values between 140—170 milli-equivalents per litre. The maximal values observed were about 180 mill. eq./lit.

*Pyloric extracts from cats*. Up to date 43 extracts from the pyloric mucosa of cats have been tested. As seen from the table, only one extract was inactive. Five caused a secretory increase beyond 2 ml in 60 minutes, and were considered to be doubtfully active. Eight extracts were classified as only slightly active (yielding 2—5 ml juice in 60 minutes), whereas 29 evoked an abundant secretion amounting to 5—25 ml in 60 minutes.

*Pyloric extracts from dogs.* The extracts from dog's pyloric mucosa were usually tested on dogs. A few have also been tested on

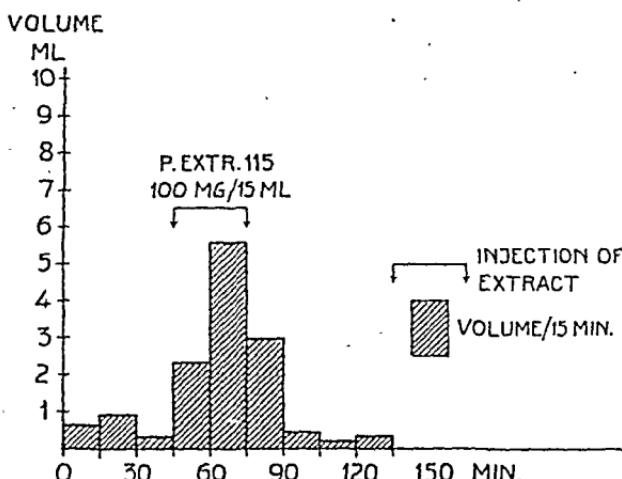


Fig. 1 a. Cat 4.2 kg. Gastric secretion during slow intravenous injection of a pyloric extract from cat.

cats. Out of twelve extracts two were inactive, two doubtfully active, and three only slightly active. Five extracts evoked a copious secretion amounting to 30—60 ml in 60 minutes.

*Pyloric extracts from pigs.* Fourteen extracts were tested on cats. Among these, one showed no activity, and four only slight activity, whereas 8 evoked an abundant secretion.

16 extracts from the cat's corpus mucosa were tested. 13 of these extracts were quite inactive, whereas 2 were doubtfully positive, and 1 induced a slight secretion.

1 extract from the dog's corpus mucosa was inactive, and of 4 similar extracts from pigs 3 were inactive and one doubtfully active. Two extracts from the cardiac mucosa of pigs were also inactive.

Some extracts from different regions of the intestines were also tested.

<sup>1</sup> The part of the stomach distally to the incisura angularis was considered to belong to the pyloric region.

VOLUME

ML

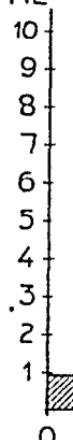
P. EXTR.  
65P. EXTR.  
66C. EXTR. P. EXTR.  
67 65

Fig. 1 b. Cat 3.6 kg. Gastric secretion during slow intravenous injection of two pyloric (no. 65 a. 66) and one corpus extract (no. 67) from pig.

VOLUME

ML

P. EXTR. 112  
60 MG/15 MLP. EXTR. 118  
30 MG/MLP. EXTR. 112  
60 MG/15ML

10  
9  
8  
7  
6  
5  
4  
3  
2  
1  
0

Fig. 2. Cat 3.6 kg. Gastric secretion during slow intravenous injection of two pyloric extracts from pig. Note the considerable decline in the secretory response to p. extr. no. 112.

Contrary to KOMAROV no activity could usually be observed in extracts from the duodenal mucosa. Only one extract from the cat's duodenal mucosa showed slight activity. Three extracts from cats, one from dogs, and three from pigs were quite inactive.

Some extracts from the mucosa of the ileum and colon of dogs and cats were also inactive.

It was frequently seen that during the course of an experiment the secretory response to an extract diminished. This was particularly seen when extracts from pigs were used. Fig. 2 shows the

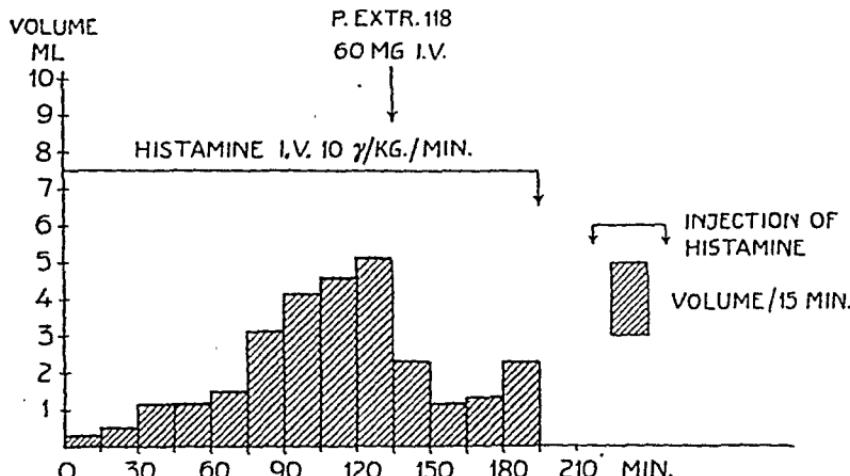


Fig. 3. Cat 3.5 kg. Gastric secretion during slow intravenous injection of histamine ( $10 \gamma'$  per kg body weight and minute). After a period of secretion 60 mg (corresponding to 5 gm moist mucosa) of a pyloric extract from pig are injected intravenously. Note the decline in the secretory rate.

considerable decline of the secretory response in an experiment where two different extracts from pig's pyloric mucosa were investigated. When testing several extracts on one animal it was therefore necessary now and then to interpose an extract of known activity. The decline in the secretory response is probably due to toxic or inhibitory influences of impurities in the extracts. That this was so was indicated by experiments as illustrated in fig. 3. A continuous gastric secretion was induced by intravenous injection of  $10 \gamma$  histamine per kg body weight and minute. An intravenous injection of 100 mg of a pyloric extract from pig (corresponding to 10 gm of moist mucosa) caused an immediate decline of the secretory rate. It is to be noted that when tested in the usual manner by slow intravenous injection the same extract caused gastric secretion.

The presence of various amounts of impurities in the extracts was also indicated by the fact that no relation was found between the amount of dry residue and the activity of the extracts.

### Mode of Administration.

In the experiment represented in fig. 4 100 mg of a pyloric extract from cat was administered intravenously, intramuscularly, and intraperitoneally. Only the intravenous injections were effec-

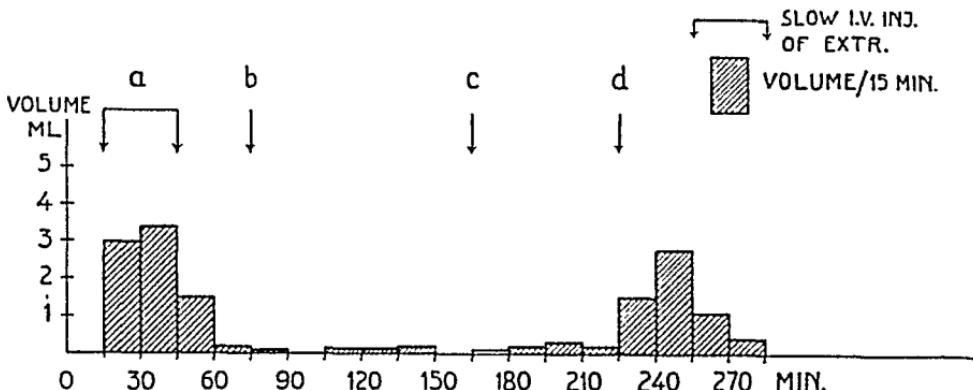


Fig. 4. Cat 3.6 kg. Gastric secretion after intramuscular, intraperitoneal, and intravenous injection of 100 mg of a pyloric extract from cat.

- a. Slow intravenous injection.
- b. Intramuscular injection.
- c. Intraperitoneal injection.
- d. Intravenous injection.

tive. Intramuscular and intraperitoneal injections induced only a very scanty secretion. It was generally seen that subcutaneous, intramuscular, and intraperitoneal administration of the extracts caused no, or a very scanty secretion. We do not know the reason for this. It is a remarkable fact that secretine too is ineffective — or nearly so — when administered subcutaneously, intramuscularly, or intraperitoneally (ÅGREN 1934, MELLANBY 1928).

### The Histamine Content of the Pyloric Extracts.

In his report KOMAROV claims that when tested biologically according to BEST and McHENRY, and BARSOUM and GADDUM his active pyloric extracts were found to be histamine free. When testing directly on the guinea-pig's ileum, Uvnäs observed that his pyloric extracts caused contractions of the intestine possibly due to traces of histamine. The histamine concentrations found were too small, however, to be made responsible for the gastric secretory effect of the extracts. When tested on the cat's blood-pressure the extracts showed no depressor effect.

We have tested most of our later extracts on the guinea-pig ileum. The technique was identical with that described by EMMELIN et al. (1941). By using this technique a histamine concentration of 20  $\gamma$  per litre is safely determined. Most of the extracts tested were found to be quite histamine-free. Some caused a slight

contraction of the intestine corresponding to only 20—30  $\gamma$  histamine per litre.

No relation was found between the contractile response to and the secretory effect of an extract. On the contrary, extracts exciting gastric secretion mostly were quite inactive when tested on histamine on the guinea-pig ileum.

EMMELIN et al. claim that the histamine found on extracting blood plasma and testing the extracts pharmacologically is present normally in the plasma in a physiologically inactive state. To exclude the possibility that the activity of the pyloric extracts might be due to histamine in such an inactive state, some of the extracts were chemically treated according to the modified Barsoum-Gaddum method used by EMMELIN et al. in their investigations on plasma histamine. No traces of histamine could be detected.

### Discussion.

Confirmatory to earlier reports by KOMAROV and UVNÄS, a gastric-secretory excitant was obtained in extracts from the pyloric mucosa of cats, dogs, and pigs. Extracts from other gastric regions were found to be inactive, or to evoke only a very slight secretion. It is possible that improved extraction technique will reveal the presence of a gastric-secretory agent also in other regions of the gastric mucosa. Anyhow, our results indicate that the pyloric mucosa occupies a special position, containing much greater amounts of the active factor than other stomach regions.

The active extracts were found to be histamine-free when tested directly on the guinea-pig ileum, as well as when tested after being extracted by a modified Barsoum-Gaddum method.

Earlier investigators, GROSS (1906), EDKINS and TWEEDY (1909), SAWITSCH and ZELIONY (1913), KLEIN (1935), UVNÄS and others in cross-circulation experiments and experiment on animals with isolated or autotransplanted stomach pouches have provided evidence in favour of the view that the humoral mechanism of gastric secretion is chiefly connected with the pyloric region. After SACNS, IVY, BURGESS and VANDOLAH had succeeded in isolating crystalline histamine from extracts of the pig's pyloric mucosa, histamine was generally accepted as "the gastric hormone". GAVIN, McHENRY and WILSON, as also later EMMELIN et al. found that the corpus mucosa of the stomach contains more histamine

Table 1.

*Gastric Secretion During Slow Intravenous Injection of Mucosal Extracts from Different Regions of the Stomach and the Duodenum.*

Animal	Mucosal region	Activity of the extracts				Total number of extracts
		strongly active	slightly active	doubtfully active	inactive	
Cat	Pyloric region	29	8	5	1	43
>	Corpus	0	1	2	13	16
>	Duodenal	0	1	0	3	4
Dog	Pyloric	5	3	2	2	12
>	Corpus	0	0	0	1	1
>	Duodenal	0	0	0	1	1
Pig	Pyloric	9	4	0	1	14
>	Corpus	0	0	1	4	5
>	Cardiac	0	0	0	2	2
>	Duodenal	0	0	0	3	3

than the pyloric mucosa. The active histamine-free factor obtained by KOMAROV and us is predominantly related to the pyloric mucosa. The pronounced localization of the excitatory factor to the pyloric region, together with the experimental evidence that the humoral mechanism of the gastric secretion is chiefly related to the same region, strongly indicate that the active agent of our pyloric extracts is identical with the gastric hormone, the existence of which was postulated by EDKINS.

### Summary.

A gastric secretory factor predominantly localized in the pyloric region is present in mucosal extracts from cats, dogs, and pigs.

The active agent is not histamine.

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## Histamine and Peptic Secretion.

By

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Received 15 May 1943.

The stimulating effect of histamine on the HCl-producing gastric glands is a well-known fact. Whether histamine also activates the peptic glands is a matter of dispute.

Among other investigators BABKIN (1930) and VINEBERG and BABKIN (1931) claim that histamine selectively activates the HCl-producing glands, while it does not stimulate the peptic glands. They found in experiments on dogs with Heidenhain or Pavlov pouches that subcutaneous administration of 0.75—1.0 mg of histamine evoked a copious secretion of a gastric juice rich in HCl. The first portions of the juice showed a considerable digestive power. The peptic activity in the following samples rapidly fell to scarcely detectable values. A second dose of histamine augmented the HCl-secretion but did not affect the peptic activity. The initial digestive power of the gastric juice secreted after histamine they considered to be the result of "a washing out" of preformed enzyme from the peptic glands. GILMAN and COWGILL (1931) arrived at the same conclusions in experiments similar to those of VINEBERG and BABKIN. In experiments on anaesthetized dogs BOWIE and VINEBERG (1935) found the gastric juice obtained after subcutaneous administration of histamine to be extremely poor in pepsin. Electrical stimulation of the vagi in the neck, when applied immediately after a period of administration of histamine, stimulated the previous inactive peptic glands, as mani-

fested by a rise in the peptic power of the juice. Histological examinations of the gastric mucosa revealed maximal discharge of pepsinogen granules from the peptic cells after prolonged electrical stimulation of the vagi. Contrary to this, several hours' subcutaneous administration of histamine caused no detectable diminution in the amount of pepsinogen granules.

The experiments mentioned above indicate that histamine does not stimulate the peptic glands. In recent years BUCHER and Ivy (1941), and BUCHER, Ivy and GRAY (1941) in experiments on dogs with vagotomized total stomach pouches have arrived at a different opinion. BUCHER and Ivy investigated the peptic secretion after a "double histamine test" and found, contrary to earlier investigators, that the peptic activity of the gastric juice rose also after a second subcutaneous histamine dose. The same authors claim that pepsin is partially inactivated already at a pH above 1.5—2. The lack of agreement between their experiments and those of others they consider as possibly due to the fact that in earlier experiments no regard had been paid to the inactivation of pepsin in gastric juice of low acidity, and that unreliable methods for the determining of the peptic activity had been employed. BUCHER and Ivy used the BEAZELL modification of the hemoglobin method of ANSON and MIRSKY. BUCHER, Ivy and GRAY administered histamine in rather small doses subcutaneously in dogs every 10 minutes. In eight-hour experiments they found no diminution of the peptic activity in the gastric juice throughout the experiment. If during the course of such an experiment the histamine dose was increased, not only the HCl — but also the pepsin output rose. From these results BUCHER *et al.* conclude that histamine stimulates the peptic glands.

Clinical investigations have given divergent results. Most investigators POLLAND (1932), RIVERS, OSTERBERG and VANZANT (1936), RIVERS and VANZANT (1937) and others claim that histamine stimulates the peptic glands in man. IHRE (1938), as also TOBY (1937) and BABKIN (1938), state that histamine has not effect on pepsin production in man, and consider the pepsin of the gastric juice secreted after histamine to be "washed out".

From experiments on dogs ALLEY (1935) states that under certain conditions histamine inhibits the pepsin secretion.

### The Author's Experiments.

All experiments were performed on cats from which food had been withheld during the preceding 20 hours. As anaesthetic a mixture of chloralose and urethane was used. During a short ether anaesthesia 0.05 g chloralose and 0.5 g urethane per kg body weight were slowly injected in the iliac vein.

*Operative procedure.* After opening the abdomen a short incision was made in the ventral wall of the stomach just proximal to the pyloric region. The stomach was drained through a thin perforated rubber tube introduced through a glass cannula inserted in the opening made in the ventral stomach wall.

The tip of the cannula and the rubber tube were then pushed through an incision in the left abdomen, and the abdominal wall was closed. To avoid regurgitation of intestinal contents the duodenum was ligated just proximal to Vater's ampulla. During the experiment the animal was placed on its left side, the front half of the body somewhat higher to facilitate the outflow of the gastric juice.

In experiments where electrical stimulation of the vagi was performed, the vagus nerves were exposed in the neck, separated from the sympathetic trunks, and cut as high as possible. The nerves were stimulated by platinum electrodes of the Sherrington type. The electrical stimulus was derived from a generator yielding alternating current of about 40 periods per second. Rhythmic alternating stimulation of the two nerves was arranged for by attaching into the circuit a metronome with a frequency of about one stroke per second.

In experiments where the vagus nerves were not stimulated the nerves were cut in the neck in order to avoid cerebral influences on the peptic glands. The trachea was cannulated and the oesophagus tied in the neck in order to prevent saliva from running into the stomach.

Histamine was slowly injected intravenously. The rate of gastric secretion was measured in 15-minute periods, and the gastric juice tested for acidity and peptic activity.

*Determination of peptic activity.* The peptic activity was determined by the hemoglobin method first described by ANSON and MIRSKY (1932) and then modified by BEAZELL et al. (1938). With suitable changes in the concentrations of gastric juice and hemoglobin and other minor modifications, we adapted the method for the Pulfrich photometer.

*The principles of the method.* An acidified hemoglobin solution is incubated with pepsin under standardized conditions. After a definite time of digestion the enzymatic process is interrupted by precipitating the undigested hemoglobin with trichloroacetic acid. The precipitate is filtered off, and the concentration of digestion products, which give a blue colour with phenol reagent (tyrosine, tryptophane and cysteine), is determined.

*Definition of peptic activity.* One unit of pepsin is defined as that quantity which acting at 37° C. in the standard digestion mixture used would liberate in 60 minutes a quantity of material soluble in trichloracetic acid, a quantity, which in the assay mixture is equivalent to one milligram of tyrosine. The peptic activity is given in peptic units per ml of gastric juice.

<i>Reagents used.</i>	Hemoglobin solution . . . . .	10 %
	Trichloracetic acid . . . . .	4 %
	HCl . . . . .	N/6
	NaOH . . . . .	3.85 N
	Phenol reagent according to Folin and Ciocalteau.	

*The preparation of hemoglobin* is carried out according to ANSON and MIRSKY, with the modification that instead of carbon monoxide, coal gas is bubbled through the blood.

*Procedure of testing.* To make up the acid solution of hemoglobin, 3 volumes of N/6 HCl are added to 2 volumes of 10 % hemoglobin solution. 5 ml of this mixture is pipetted into a test tube and heated in a water bath at 37° C. for 15 minutes. 0.2 ml of gastric juice are then added. After electrical stimulation of the vagi gastric juice is generally diluted 5 times, whereas histamine juice and other juices poor in pepsin are used undiluted. After 15 minutes the digestion is interrupted by adding 10 ml of 4 % trichloracetic acid, and after careful mixing the precipitate is filtered off. 3 ml of the filtrate are pipetted into a glass vessel containing 20 ml of distilled water, and 1 ml of 3.85 N NaHO and finally 1 ml of phenol reagent are then added. Readings are made in the Pulfrich photometer after 15 minutes. After this time no colour change occurs within 1—2 hours. Cuvette length 10 mm and filter S 57 are used. As the gastric juice in itself gives a slight colour reaction with phenol reagent, all samples are checked against a compensation solution. This contains the same ingredients as the other samples except that the gastric juice is inactivated. The pepsin is destroyed by boiling 5 minutes in a water bath.

According to BUCHER and BEAZELL (1941) the gastric juice contains inhibitory substances, which influence the peptic digestion. This inhibitory effect varies with the degree of dilution of the gastric juice. This fact is to be considered in experiments where great variations of peptic activity make it necessary to use different concentrations of the gastric juice.

*Determination of acidity.* Total HCl was determined by electro-metrical titration, a potentiometer according to Prahn being used. PH 7.4 was taken as the limit for total HCl.

## Results.

*Volume and acidity.* Slow intravenous injection of histamine initiated and maintained, as previously shown by many observers,

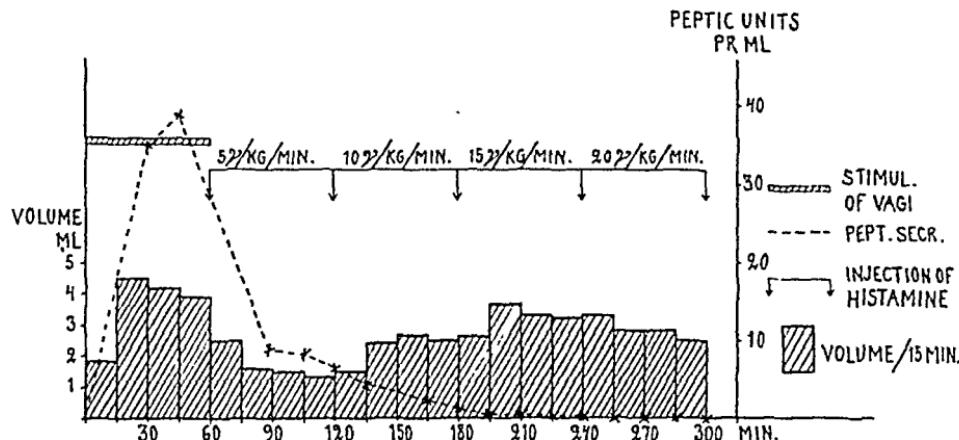


Fig. 1.

secretion of a gastric juice of high acidity. The secretion began ca. 15—30 minutes after starting the histamine injection. The rate of secretion rose successively during the first 30—60 minutes, and then tended to be constant during several hours' injection. The acidity also increased during the first periods, and then usually maintained a constant value throughout the experiment. The total acidity varied in different experiments, the maximal values reaching about 150 milli-equivalents per litre. The pH of the gastric juice only exceptionally exceeded 1.5, and then in the beginning of an experiment. The rate of secretion initiated by histamine varied. In most animals 5—25  $\gamma$  histamine biphosphate per kg body weight and minute intravenously maintained a secretion of about 2.5—5 ml of gastric juice per 15 minutes.

*Peptic activity.* The peptic activity of the juice secreted under histamine injection was low. During the course of the secretion the digestive power decreased to extremely low or undetectable amounts. In about 20 experiments it was generally observed that *the greater the rate of secretion the earlier the juice lost its digestive power.*

In the experiment seen in fig. 1 the gastric glands respond to 60 minutes' electrical stimulation of the vagi with the secretion of a juice of high acidity and relatively high peptic activity. After this, histamine is administered intravenously. Every 60 minutes the dose of histamine is augmented, and doses from 5 to 20  $\gamma$  histamine per kg body weight and minute are used. A gastric juice of high acidity is obtained. The peptic power, however, successively falls throughout the experiment, and *the*

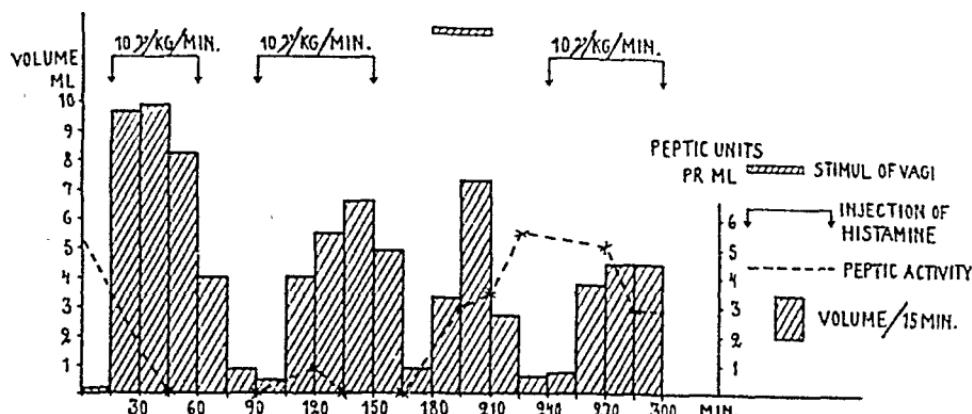


Fig. 2.

peptic cells are not stimulated by the augmentation of the histamine doses. This was invariably seen in all experiments of this kind.

In the experiment represented in fig. 2, 10  $\gamma$  histamine per kg body weight and minute are injected intravenously. During the following secretion the peptic power of the juice rapidly falls. After 45 minutes the injection of histamine is stopped and the secretion declines. *In spite of the diminishing secretion volume the pepsin concentration does not increase*, indicating that the peptic content of the glands is completely washed out. A new period of histamine injection is now started. Traces of peptic activity are seen at the beginning of the secretion, but afterwards no pepsin is detectable. After 60 minutes the histamine injection is again stopped. When the secretion has declined the vagi are stimulated electrically for half an hour. A considerable secretion of a juice of high acidity and a peptic power as high as at the beginning of the experiment is obtained. After 30 minutes' rest a new histamine injection is initiated. *The juice now obtained contains pepsin.* The difference in the digestive power of the juice secreted after the second and the third histamine injection period is striking. In the former case the peptic glands are apparently emptied of their pepsin content by a previous histamine administration. In the latter the peptic glands are activated by electrical stimulation of the vagi, and pepsin stagnates in the glands when the secretion of HCl declines. The stagnated pepsin is then washed out by the following secretion initiated by histamine.

A similar experiment is seen in fig. 3. 10  $\gamma$  histamine per kg body weight and minute are administered. A moderate secretion

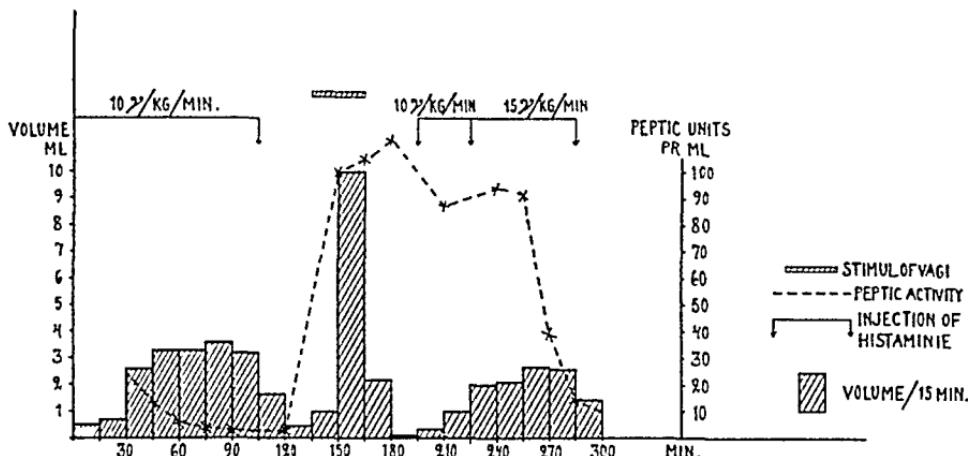


Fig. 3.

of gastric juice is maintained. The peptic power successively declines. After this the vagi are electrically stimulated for 30 minutes. A gastric juice very rich in pepsin is secreted. After the secretion has stopped histamine is injected again. *The peptic power now remains high for about an hour, but then rapidly declines.*

These experiments do not favour the view that histamine stimulates the peptic glands. They seem to indicate that *the peptic power of gastric juice secreted under histamine is dependent upon the pepsin content of the peptic glands at the beginning of the secretion*. To investigate the possibility that the low peptic content of the histamine juice was not due to inhibitory influences of histamine, the following experiments were performed.

The vagi were electrically stimulated for 60 minutes. The peptic activity of the gastric juice obtained was determined. After 30 minutes' rest histamine was administered. 60 minutes later when the secretion under histamine was continuing, the vagi were concomitantly stimulated. Fig. 4 shows such an experiment. Vagal stimulation gives a juice of high peptic activity. During the following histamine injection the peptic power falls to near zero. When stimulation of the vagi is repeated the pepsin content rapidly increases. The amounts of pepsin in the juice of the different periods of vagal stimulation are practically the same. Ten experiments of this kind revealed *no inhibitory effect of histamine on the peptic secretion following electrical vagal stimulation*. Histamine doses between 5—25 γ per kg. body weight and minute were employed.

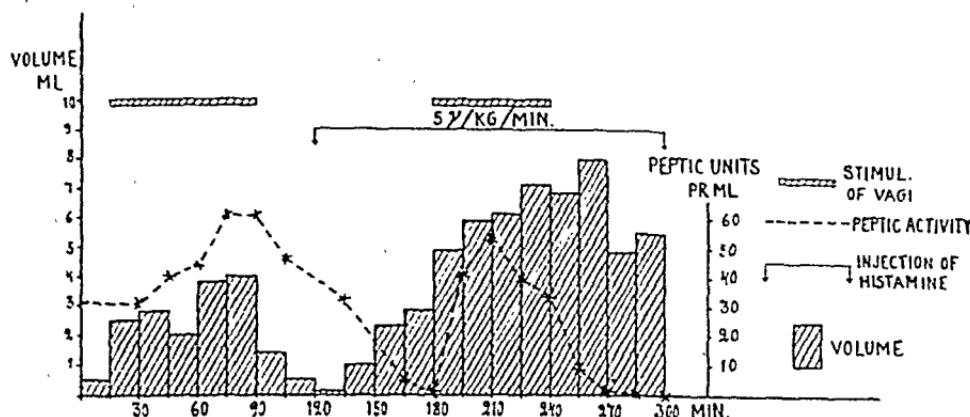


Fig. 4.

### Discussion.

Our experiments have shown:

- a) That slow intravenous injection of histamine yields a gastric juice of high acidity but of low peptic activity.
- b) That the peptic activity continuously falls during the gastric secretion, and that there exists a reciprocal relationship between the rate of the HCl secretion and the peptic output. The greater the secretion of HCl, the more rapid the fall of digestive power.
- c) That augmenting the histamine dose does not influence the peptic output. The fall of digestive power continues.
- d) That in a "double histamine test" the second histamine dose yields a gastric juice practically pepsin free. If, however, a period of electrical stimulation of the vagi is interposed, the histamine juice again shows peptic activity.

These results do not favour the view that histamine stimulates the peptic glands. They rather support the "washing out" theory, and indicate that the pepsin content of the gastric juice secreted after histamine is dependent on the amount of preformed pepsin in the glands.

Our results thus agree with those of BABKIN and BABKIN and VINEBERG, as also with those of VINEBERG and BOWIE. They are contrary to those of BUCHER and IVY, and BUCHER, IVY and GRAY. BUCHER and IVY refer the lack of agreement between their results and those of other investigators to the fact that in earlier experiments no regard had been paid to the inactivation of pepsin at pH above 1.5—2, and that unreliable methods for

pepsin determination had been employed. In our experiments the pH of the gastric juice has not exceeded 1.5, and like these authors we have used the hemoglobin method for determining the peptic power of the juice. The arguments of BUCHER and IVY are thus not applicable to our results. The divergence between our results and those of BUCHER, IVY and GRAY may possibly be due to other factors. In an unanaesthetized dog a continuous basal secretion of pepsin is likely to occur. In their experiments BUCHER, IVY and GRAY administered "submaximal histamine doses". The secretion thus obtained is possibly not sufficient to "wash out" the pepsin continuously formed in the glands.

### Summary.

Histamine does not stimulate the peptic glands of the cat's gastric mucosa.

Histamine does not inhibit the peptic secretion evoked by electrical stimulation of the vagi.

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## Some Chemical Properties of the Gastric Secretory Excitant from the Pyloric Mucosa.

By

BÖRJE UVNÄS.

Received 21 June 1943.

KOMAROV (1938) reported the existence of a gastric secretory excitant in HCl-extracts from the pyloric mucosa of dogs. In experiments on dogs and cats Uvnäs (1942, 1943) adduced strong evidence in favour of the view that a pyloric hormone plays a part in the physiological mechanism of the gastric secretion. The active agent is not histamine. As it was found in the protein-fraction of the extracts, it was supposed to be protein-like in nature. Further evidence supporting this idea, as far as we know, has not been brought forward.

### Experimental.

The experiments were carried out on anaesthetized cats, the technique devised by Uvnäs (1943) being used.

The vagi were exposed in the neck and cut. The trachea was cannulated and the oesophagus tied in the neck. The stomach was drained by a thin perforated rubber tube introduced into the stomach through a glass cannula inserted in the stomach wall just proximal to the pyloric region. The cannula with the rubber tube was pushed out through an incision in the left abdomen. The duodenum was ligated just proximal to Vater's ampulla. After the abdominal wall was closed the animal was placed on its left side, the front half of the body somewhat higher to facilitate the outflow of the gastric juice.

The extracts to be tested were suspended in Tyrode solution made slightly acid with N/10 HCl. An opalescent solution was obtained.

On testing extracts from cats an amount of dry substance corresponding to 2—5 gm of moist mucosa was suspended in 15 ml of Tyrode solution. In testing extracts from pigs amounts corresponding to 5—10 gm mucosa were used.

The extracts were injected intravenously in the iliac vein at a rate of 0.4 ml per kg body weight and minute. After 30 minutes the injection was stopped and the secretion allowed to decline to the basal level before a new extract was tested. This was usually the case within 30 minutes after the end of the injection.

The secretory volume was measured in 15-minute periods. The secretion during the 60 minutes following the beginning of the injection was taken as a measure of the activity of the extracts. The total acidity of the gastric juice was determined colorimetrically, phenolphthalein being used as indicator and N/10 NaOH as base.

As remarked by Uvnäs (1943) it was frequently seen that during the course of an experiment the secretory response to the extracts diminished. When testing several unknown extracts on the same animal, it was therefore necessary now and then to interpose an extract of known activity.

*Preparation of extracts.* The pyloric mucosa was removed, minced with scissors or ground in a mincing-machine, suspended in 10 volumes of N/10 HCl, and heated for 30 minutes at 100° C. in a water bath. The acid extract was then left in the refrigerator over night. Next morning, after centrifuging and filtering through cotton wool, the filtrate was neutralized with N NaOH to pH 4—5, and precipitated with an equal volume of 10 % trichloracetic acid. 1—2 hours later the precipitate was centrifuged off and washed 3 times with 40—50 volumes of 10 % trichloracetic acid in saline, twice with 50 volumes of acetone, once with benzene, twice with ether, and dried at 37° C. The white powder obtained was then stored in the refrigerator.

## Results.

*Solubility.* The active agent is soluble in water. The following observation indicates that the solubility is dependent on the pH of the solution. When neutralising the HCl-extracts beyond pH 4—5, a precipitate was formed. When resolved in N/10 HCl this solution showed excitatory properties. Quantitative determinations were not carried out, but the observations made seem to indicate that the solubility of the active agent has a minimal value between pH 6—8.

The excitatory factor is not soluble in ether, acetone, benzene, or absolute alcohol; and, contrary to the report of KOMAROV, it also seems to be insoluble in 80 % ethyl alcohol.

*Stability.* Stored in the refrigerator, the dry extracts still showed considerable activity after a year. Suspended in slightly

acid, neutral, or slightly alkaline saline at 20° C. the activity remained unaltered for at least 24 hours. As seen from the table, the agent is stable when heated for 30 minutes in N/10 HCl at 100° C. Similar heating in N/10 NaOH, however, results in complete destruction.

#### *Action of enzymes:*

*Pepsin.* An amount of extract was suspended in 15 ml of Tyrode solution at a pH of about 2, and treated for 30 minutes at 37° C. with  $\frac{1}{2}$  ml of a commercial pepsin preparation. Control samples without pepsin were treated in the same way. As seen from the table, the active agent in all experiments was partially destroyed by pepsin.

*Duodenal juice.* As a pure trypsin preparation was not available, the extracts were treated with duodenal juice, obtained from the cat's duodenum after intravenous injection of 5 mg secretin.<sup>1</sup> 15 ml of an extract was digested for 30 minutes by  $\frac{1}{2}$  ml duodenal juice at a pH of about 8 and at a temperature of 37° C. As seen in the table, no activity remained after 30 minutes digestion. Probably the inactivation of the extracts was due to the trypsin content of the duodenal juice. Control experiments without duodenal juice showed no diminution of the activity of the extracts.

#### *Action of ultraviolet light.*

Hormones of protein nature such as secretin and insulin are inactivated by ultraviolet light (ÅGREN 1934). We investigated the influence of ultraviolet light on the activity of our extracts in the following way. 15 ml of an extract solution in Tyrode was exposed to the radiation of a Hanau-quartz lamp at a distance of 10—20 cm. The temperature of the solution during the experiment did not exceed 20° C., cooling being obtained by means of an air current. The table shows the considerable inactivation after 5 hours radiation.

*Dialysis.* 15 ml of an extract solution was dialysed at 15° C. against 5 litres of saline, a cellophane membrane freely permeable to crystalloids but impermeable to hemoglobin being used. Most of the active agent passed through the cellophane membrane in 18 hours.

<sup>1</sup> Pancreotest (Astra).

Table 1.  
*The activity of pyloric extracts.*

Extr. no.	Secretory volume in ml		Treatment
	after treatment	control	
80 (from cat) . . .	8.0	7.7	Extracts treated with N/10 HCl at 100° C during 30 minutes.
108 ( " pig) . . .	2.0	3.3	
115 ( " cat) . . .	21.9	14.1	
165A ( " ) . . .	5.1	4.2	
169A ( " ) . . .	6.2	5.5	
80 ( " cat) . . .	0	7.0	Extracts treated with N/10 NaOH at 100° C during 30 minutes.
115 ( " ) . . .	0	7.7	
159 ( " ) . . .	1.5	25.3	
165A ( " ) . . .	0	4.2	
169A ( " ) . . .	0.4	5.5	
65 ( " pig) . . .	0	2.1	Extracts treated with pepsin at 37° C during 30 minutes.
80 ( " cat) . . .	0	8.4	
108 ( " pig) . . .	1.1	3.3	
115 ( " cat) . . .	2.3	9.5	
115 ( " cat) . . .	0	24.1	Extracts treated with duodenal juice at 37° C during 30 minutes.
115 ( " ) . . .	0	9.5	
123 ( " ) . . .	0.3	6.5	
115 ( " ) . . .	1.9	14.1	Extracts treated with ultra-violet light during 30 minutes.
144 ( " ) . . .	0.6	8.9	
152 ( " ) . . .	0	10.6	
159 ( " ) . . .	0	10.4	
162 ( " ) . . .	0	13.1	
65 ( " pig) . . .	1.8	8.0	Extracts dialysed through cellophane during 18 hours.
80 ( " cat) . . .	4.9	11.7	
108 ( " ) . . .	1.2	4.9	

*Precipitation.* From the HCl-extract the active agent could be precipitated by 5 % trichloracetic acid, 1—2 % metaphosphoric acid, 20 % NaCl, and 5 volumes of acetone.

### Discussion.

The gastric-secretory excitant of the pyloric mucosa is found in the protein fraction of HCl-extracts precipitated by trichloracetic acid, metaphosphoric acid, NaCl, and acetone. The active agent is destroyed by pepsin, trypsin (duodenal juice), and ultra-violet light.

These facts indicate that the active substance is of protein nature.

The factor is stable when heated at 100° C., and thus is not a ferment. It passes through cellophane, indicating that it may be a relatively small molecule.

Physiologically the secretory excitant causes the secretion of a gastric juice rich in HCl but of a low peptic activity(unpublished observations).

The preparations now available are too crude to permit valid conclusions about the chemical nature of the active agent. In the intestinal canal, however, we already know one hormone, secretin, which chemically as well as physiologically shows great similarities with our gastric secretory excitant. Like the gastric excitant secretin is destroyed by pepsin, trypsin, and ultraviolet light (BAYLISS and STARLING 1902, MELLANBY and HUGGET 1926, ÅREN 1934). It is relatively stable in weak acids and alkalis (BAYLISS and STARLING, MELLANBY and HUGGET, ÅREN), and dialyses through parchment paper (BAYLISS and STARLING).

According to MELLANBY and HUGGET (1926) and to LAGERLÖF (1942) secretin selectively stimulates the pancreatic cells to produce a copious flow of sodium bicarbonate solution. The secretion of the pancreatic enzymes is considered to be under the control of the vagus nerves. In this respect secretin resembles the pyloric factor which stimulates the gastric glands to produce a copious flow of a HCl-solution of low enzyme content.

The facts available about the chemical and physiological properties of the gastric-secretory excitant of the pyloric mucosa thus make it possible that it is a substance chemically related to secretin. According to ÅREN this is a protein-like substance with a molecular weight of about 5,000.

### Summary.

The gastric-secretory excitant from the pyloric mucosa shows the following chemical porperties: —

It is soluble in water. The solubility varies with the pH.

It is insoluble in ether, acetone, benzene, and absolute and 80 % ethyl alcohol.

It is stable in weak acid and alkaline solutions. It resists heating in N/10 HCl at 100° C. but is destroyed when heated in N/10 NaOH to this temperature.

It is inactivated by pepsin, trypsin (duodenal juice), and ultraviolet light.

It dialyses through cellophane.

It is precipitated by trichloracetic acid, metaphosphoric acid, NaCl, and acetone.

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## Correlation between Changes in Cross Striation and Mechanical Tension in Striated Muscle Fibre and their Structural Interpretation.

By

FRITZ BUCHTHAL and G. G. KNAPPEIS.

Received 17 May 1943.

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An analysis of the structural changes corresponding to the mechanical reactions of the cross striated muscle fibre, is a link in the effort of understanding the process of contraction.

The aim of the present work is to correlate the changes in length of the anisotropic (A) and the isotropic (I) substance during isometric contraction, with the mechanical tension produced by the fibre.

Previous investigations (insect muscles, HÜRTHLE, 1909, frog muscles, BUCHTHAL, KNAPEIS and LINDHARD, 1936), have shown that a marked change is found in the proportion between the anisotropic and the isotropic substance at rest and during contraction, but the time course of the change and its relation to the extent of the tension developed have hitherto not been investigated.

In a preceding investigation (BUCHTHAL and KNAPEIS 1943), simultaneous microcinematography of two points of the fibre revealed a phase difference in the course of the changes in cross striation, indicating a propagation of these changes at a rate of 0.3 m/sec (20° C.). Further, a characteristic time course for the changes in cross striation of the single fibre element (compartment), was found.

In the present work, a quantitative examination of the changes in cross striation is carried out simultaneously with the regis-

tion of mechanical tension as a function of time, at different strengths of stimulation and different degrees of stretch.

It seems reasonable to expect that a grading in the changes in cross striation corresponds to a gradation of the contraction tension developed. It may, however, already be stated here that the results found are not in agreement with this supposition.

## Method.

### 1. Preparation.

The experiments are performed on single fibres of *m. semitendinosus* (*Rana esculenta* and *Rana temporaria*), which are prepared under the microscope from tendon to tendon in an ice-cooled Ringer solution. The frogs are curarised by an injection of  $0.5 \gamma/g$  Curarine, the effect

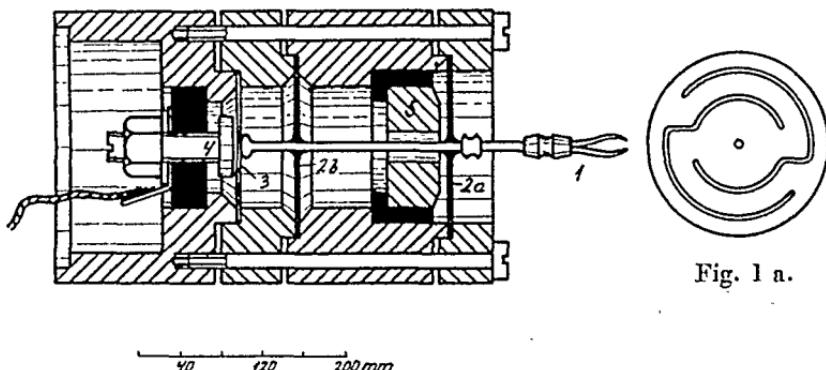


Fig. 1 a.

*Fig. 1. Condenser Myograph.* (1) micro-tweezer for attachment of one tendon end of the muscle fibre. (2a) and (2b) spring plates to hold (1) in place. (cf. detail in Fig. 1a). (3) aluminised mica membrane, (4) condenser plate, distance from (3) being variable. (5) electrode which produces an electric field between (5) and (2a) for mechanical calibration.

being controlled by stimulation of the sciatic nerve. The pH of the Ringer solution, after a stream of 99 per cent  $O_2$  and 1 per cent  $CO_2$  has been passed through it for 5 minutes, is 7.3—7.4, and the pH value throughout the experiment does not vary.

To ensure a suitable colloid-osmotic pressure, the Ringer solution contains, besides salts and glucose, 6 per cent dialysed Gum. arabic. or 1.35 per cent Polyviol Am.

The fibre is fastened by its tendon ends to two micro-tweezers (Fig. 1 (1) — the other tweezer is not included in the diagram), and is placed in a chamber filled with Ringer solution, which is closed with a cover glass so that, throughout the experiment, it lies in Ringer solution (temperature 18—20° C.).

## 2. Stimulation.

The stimulation is led to the fibre from a thyratron generator over the two stainless steel tweezers. The stimulus is a rectangular current pulse, the intensity of which can be varied at will, and the duration of the stimulus is 1 ms. The moment of stimulation is marked on the film by means of an electrostatic oscillograph connected with the stimulation device.

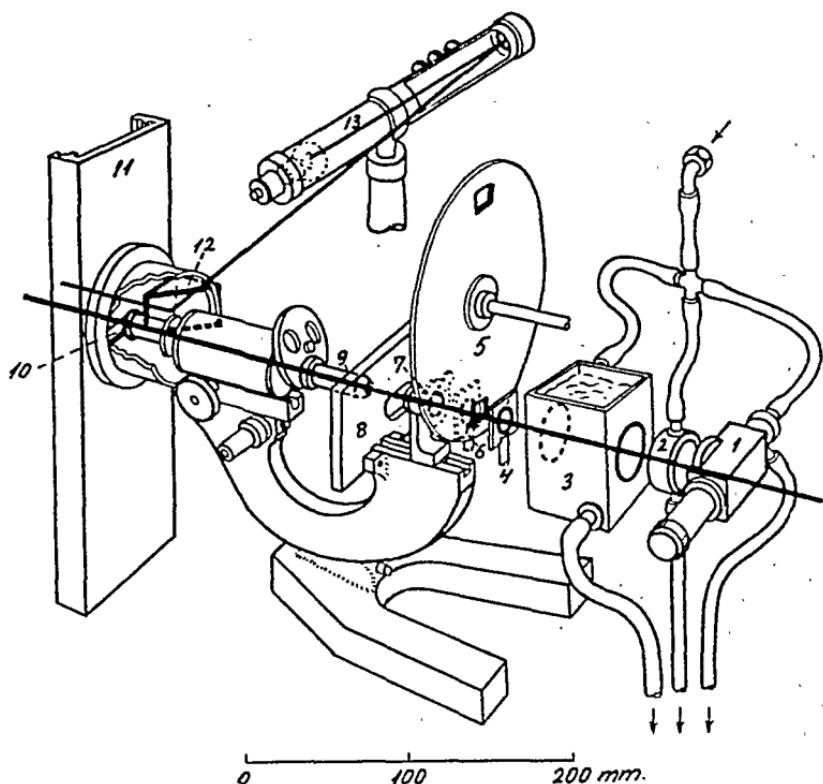
In a series of experiments where the tension, initiated by local stimulation of one end of the fibre, is compared with that resulting from a lengthwise stimulation of the whole fibre, micro-electrodes and a micro-manipulator are used.

## 3. Tension Registration.

The changes in cross striation are registered microcinematographically by an arrangement fully described in a previous paper (1943). The mechanical tension is recorded as changes in capacity, and the arrangement corresponds in principle, to that of the myograph formerly described (BUCHTHAL 1942). The tension arising from stretch and contraction displaces one movable plate of a condenser. The arrangement previously used proved unsuitable for the present purposes, because of its high sensitivity to mechanical vibration, caused mainly by dynamic forces originating from the synchronous motor which drives the rotating disc ((5) Fig. 2). It was, therefore, necessary to construct a measuring device, which, with the same stiffness, had considerably less mass in the movable system. Fig. 1 shows the condenser arrangement in section. The fibre tension is transferred to an aluminised mica plate (3), which is hereby subjected to elastic deformations by which its distance from an amber-isolated adjustable plate is varied and the desired change in capacity obtained. The tweezer is held in place by two spring plates (2a and 2b). Fig. 1 a shows these spring plates which ensure an axial movement of the tweezers. When working the tweezers, the spring plates and mica membrane are protected by a clamp. Opposite the spring plates (2a), an electrode (5) is placed in amber-isolation. When electric tension is laid upon it, an electric field arises between the electrode and the spring plate. The mechanical tension produced hereby is calibrated once and for all with a spring balance and the tension produced by the electric field on (5), is used in the experiment as mechanical calibration.

The variations in capacity are led via a shielded cable of a low and constant capacity (BUCHTHAL and WARBURG 1943) to a high frequency arrangement described elsewhere (BUCHTHAL 1942).

The resonance frequency of the condenser arrangement amounts to 700—800 vibrations per second, and the frequency curve is well suited to the purpose. Over the range of frequency in question for the registration of single contractions (10—100 cycles/sec), a distortion by mass or damping of the system is of no importance.



*Fig. 2. Arrangement for microcinematographing cross striation and for recording tension.* (1) water-cooled super high-pressure mercury lamp (Philips "Philora" sp. 500, brightness 33,000 Stilb, luminous flux 15,000 lumen); (2) water-cooled collector, (2 lenses of 20 dioptr.); (3) cooling trough; (4) collecting lens; (5) rotating disc with 2 slits, 3,000 rev./min. corresponding to 100 pictures/sec, (6) collecting lens; (7) microscope condenser; (8) microscope stage with condenser myograph and muscle chamber; (9) microscope objective (Zeiss Epi 40  $\times$ , N. A. 0.65); (10) projection eyepiece 9  $\times$ ; (11) fall camera; (12) totally reflecting prism for the projection of the course of mechanical tension and the base line with stimulation marking on the film; (13) electrostatic oscillosgraph for tension recording.

In a high frequency circuit, the changes in capacity are transformed to electric tension which is amplified in a direct current amplifier, and registered by an electrostatic oscillosograph (Fig. 2 (13)), the oscillations of which are projected through a totally reflecting prism (Fig. 2 (12)), on the film strip. The indication of the stimulus is recorded in a similar manner, but the oscillosograph used for it is omitted in Fig. 2.

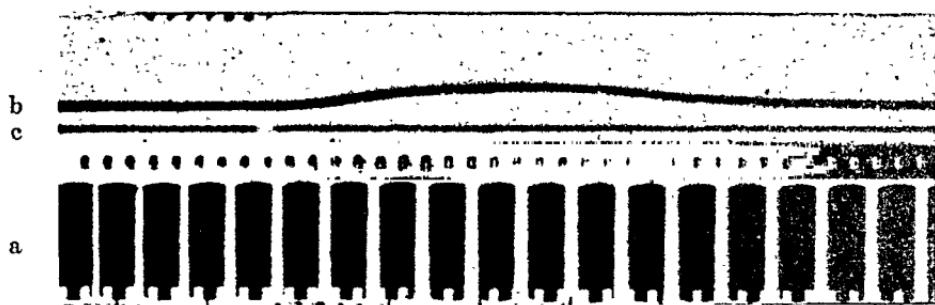
#### 4. Microcinematographic Registration.

The changes in cross striation and the mechanical tension are recorded on the same film strip and the optical arrangement is seen in Fig. 2. The light from a water-cooled high-pressure mercury lamp is collected by a system of water-cooled lenses (2, 3, 4) on the condenser (7) of the microscope. (9, 10.) The rotating disc allows the light to pass

with a frequency of 100 pictures/sec and an exposure of 0.4 ms. For photographic recording a fall camera is used. Rate of fall 1 m/sec.

The exposure should not exceed c. 5 ms, and it is necessary that the cross striation runs with the direction of movement of the film. The finite time of exposure causes a certain blurring of the photograph, whereby its evaluation would become impossible if it occurred across the cross striation. It is, however, of no importance for measuring A and I when the cross striation lies in the direction of movement of the film, as is the case when the fibre is placed horizontally between the tweezers on the movable stage of the microscope.

The stimulation is released by the falling film. Fig. 3 shows a film strip thus registered with a series of microphotographs (a), the mutual



*Fig. 3. Film strip with a) microphotographs of the muscle fibre, (b) simultaneously recorded mechanical tension, (c) base line with stimulation marking.*

time distance of which is 10 ms, and the simultaneously recorded mechanical tension (b), whith the zero line (c), and the marking of the stimulus. To indicate clearly the moment of stimulation, the duration of the marking, not that of the stimulus itself, is increased by inserting a condenser.

The film strips used in the fall camera are about 30 cm long and two strips of normal cinematographic film are glued together (negative material: Agfa Tone, negative film "Special"). The distance from the eye-piece to the film is 5 cm and the single microphotograph reduced by a rectangular diaphragm to a size 10 × 20 mm. Thirty microphotographs and the mechanical tension are recorded on one film strip.

##### 5. Measurement of the Negatives.

The microphotographs are measured on the negative under the microscope with an eye-piece micrometer with movable cobweb having a magnification of 12.5 times. The total magnification thus amounts to 900 ×. To obtain the height of compartment, the length of 10 successive compartments is measured. Furthermore, 10 measurements of the A and I substance are performed on each negative.

The course of the mechanical tension is determined under a measuring microscope with movable body tube.

## Results.

### 1. Changes in Cross Striation during Contraction as Function of Time.

The course of the changes in the anisotropic and the isotropic substances during an isometric twitch expressed by  $\frac{A}{A+I}$  as a function of time, is seen in Fig. 4. A's part in the height of compart-

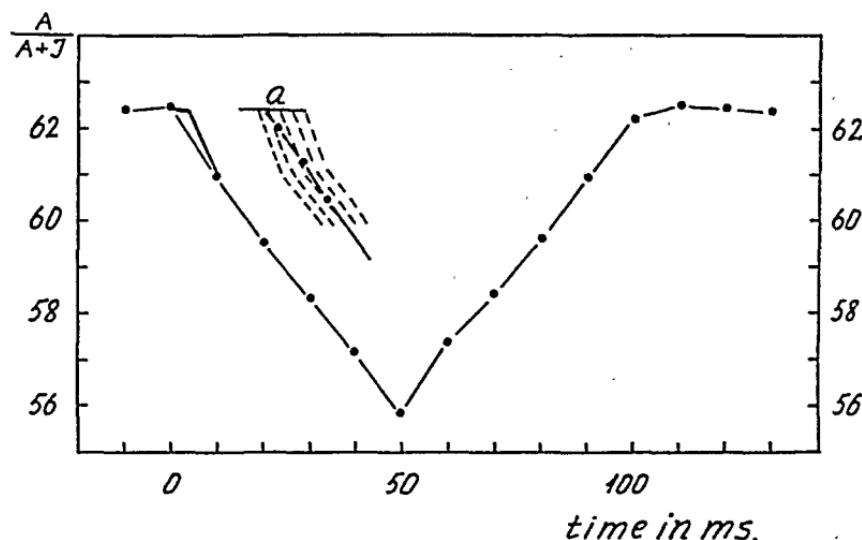


Fig. 4. Mean curve showing the variation of cross striation expressed by  $\frac{A}{A+I}$ .

Ordinate:  $100 \times \frac{A}{A+I}$ , abscissa: Time ms.

4 a Synthesis for determining the probable initial course of the curve — — — the supposed course — — mean of these curves corresponding to the curve in Fig. 4.

ment varies from 63.5 per cent at rest, to 56.0 per cent during contraction, after which it returns to the original value. The error in measurement for a single determination, amounts to 0.6 per cent of the height of compartment. The maximal change in

$\frac{A}{A+I}$  occurs (at about  $20^\circ C.$ ) 50 ms after the last resting point, and the return to the original value takes a further 50 ms. The curve in Fig. 4 represents a mean of all the experiments which are synchronised in reference to the last resting point. As a picture is recorded every tenth millisecond, and, as a higher picture

frequency was difficult to obtain, the probability of an exposure of the actual point of inclination is small. It is, therefore, not permissible to connect the last rest determination with the first point recorded during contraction, as we do not know how long rest continues in the 10 ms which elapse between the last resting point and the first recorded under contraction.

The synchronisation of the curve involves a maximal error of about 5 ms (it cannot, for example, be 6 ms, as the synchronisation in such a case would change 10 ms). The transition from rest to contraction is rounded, due to the spread of the synchronisation. The mean curve found, may, as we do not consider the error of  $\frac{A}{A+I}$  in this connection, be regarded as that of a system of congruent curves with a common level of origin, evenly distributed over a maximal time distance of 10 ms. To discover the real time course in the interval between rest and the first determination during contraction, the shape of the curve must be found, which, with the stated error in time, gives, when smoothed, the closest approximation to the mean curve for the time course of  $\frac{A}{A+I}$ . The dotted lines in Fig. 4 a show the assumed course of  $\frac{A}{A+I}$  as a function of time. The continuous curve is their mean, which is found to correspond to the gradient of Curve 4. The actual course of  $\frac{A}{A+I}$  as a function of time, must, after this, be regarded as being steeper immediately after the last point of rest than during the remainder of the fall to the minimum, and must, in its gradient, correspond to the assumed basic curve. The result of the synthesis shows that the changes in cross striations begin 3—4 ms later than a direct connection of the determined points indicates.

This correction is not only warranted, but necessary, as the latent periods for the change in  $\frac{A}{A+I}$ , in spite of a certain propagation time included in the latent period, have, in many cases, a negative value when the uncorrected curve is used as a basis for these determinations. A corresponding correction applies to the apex of the curve and to the transition from contraction to rest, but here the correction is of no practical importance. As regards

the apex, the result shows that it lies about 0.5 per cent higher. For the transition from contraction to rest, the correction would mean a sharper inclination than the directly connected points indicate, but the correction is less than that at the beginning of the curve, because this part of the time course is more rounded.

The course of the fall and rise shows a striking conformity in spite of the essential differences between the simultaneously recorded tension increase and its relaxation. The change in length

of  $\frac{A}{A+I}$  is apparently unaffected by viscosity — a factor which is discussed in more detail (p. 139).

## 2. Influence of Stretch on the Time Course of $\frac{A}{A+I}$ during Contraction.

The course of  $\frac{A}{A+I}$  at different degrees of stretch (length 100—140) as a function of time is represented in a three-dimensional co-ordinate system in Fig. 5. The time for the change is 100 ms for all degrees of stretch examined, i. e. is independent of the elongation. The duration of the fall and rise is also indepen-

dent of the degree of stretch. The change  $\frac{A}{A+I}$  (rest) —  $\frac{A}{A+I}$  (contraction) is, as in tetanic contraction, (BUCHTHAL, KNAPEIS and LINDHARD 1936, BUCHTHAL 1942), independent of stretch.

On the other hand,  $\frac{A}{A+I}$  at rest is somewhat dependent on the degree of stretch, as A's part in the height of compartment in per cent enlarges with an increasing degree of stretch. This is caused by a slight difference in the elasticity moduli of A and I. It may be mentioned that different experimental series showed

slight differences in the changes of the quotient  $\frac{A}{A+I}$  (rest), by elongation (BUCHTHAL, KNAPEIS and LINDHARD 1936, BUCHTHAL 1942, and the present material). The tendency in the different materials, however, is the same.

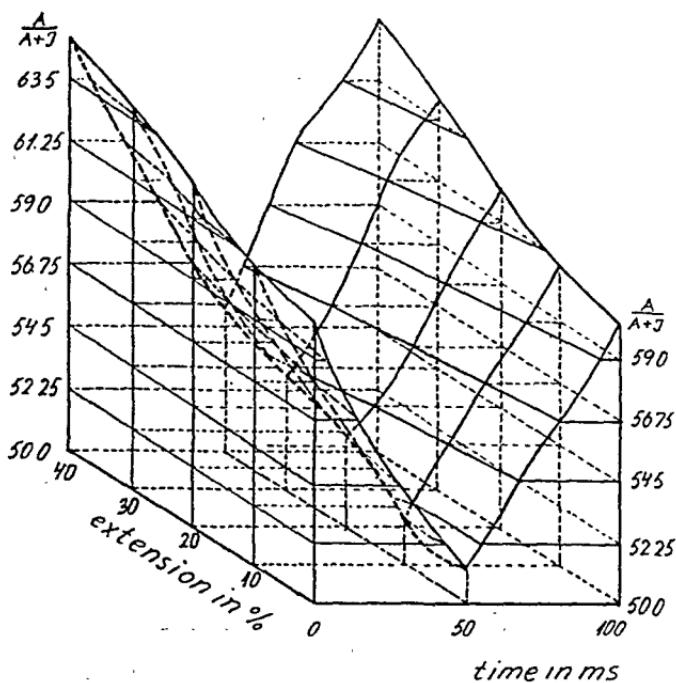


Fig. 5. The changes in the cross striation  $\left(\frac{A}{A+I}\right)$  as function of stretch and time in a 3-dimensional co-ordinate system.

Ordinate:  $100 \times \frac{A}{A+I}$ .

Abscissa I: Time in ms.

Abscissa II: Elongation in per cent.

### 3. Simultaneous Recording of Cross Striation and Tension.

Fig. 6 shows  $\frac{A}{A+I}$  as a function of time with simultaneous recording of tension after maximal stimulation. The minimum in the quotient  $\frac{A}{A+I}$  comes 50 ms after the last resting point, while the top tension is reached 70 ms after this point. 100 ms after the last resting point, the change in the cross striation has returned to its original value, while the mechanical extra-tension is still about 85 per cent of the top tension. After a lapse of 150 ms, the tension in the experiment under consideration (the mean curve of 5 experiments), has fallen to half the top tension. Furthermore, on account of the propagation, the beginning of the change of the cross striation is recorded later than the mechanical tension, as the tension already begins to develop when the fibre

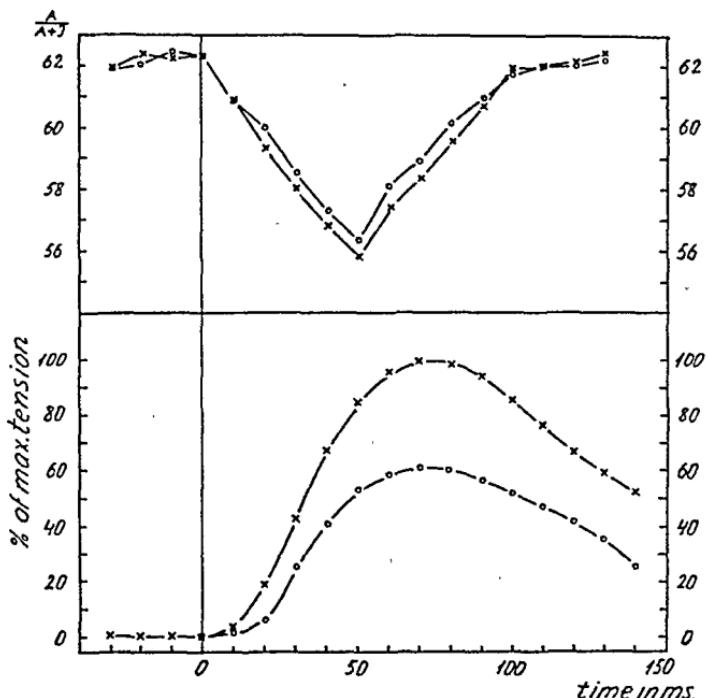


Fig. 6. Changes in the cross striation and tension as function of time in isometric single contraction.

above: change in cross striation.

Ordinate:  $100 \times \frac{A}{A+I}$ .

below: mechanical tension,

Ordinate: extra-tension of fibre in per cent of the maximal extra-tension.

Abscissa: time in ms, temp. 18°.

$\times - \times - \times -$  mean curve of the non-fatigued fibre at maximal stimulation.

$\circ - \circ - \circ -$  mean curve of fatigued fibre at maximal stimulation.

elements contract at the point of stimulation, while a change in the cross striation is first ascertained when the wave of excitation passes the point of measurement on the fibre. The point of mea-

surement for the changes in lengths of  $\frac{A}{A+I}$  cannot, for technical reasons, coincide with the point of stimulation. The delay of the maximum of the extra tension has various causes:

1. The propagation — the influence of which is dealt with in a previous communication (BUCHTHAL, and KNAPEIS 1943), and

2. The contraction remainder or viscosity of the fibre, which must be equally distributed over  $A$  and  $I$  as it is not visible in the quotient  $\frac{A}{A+I}$ .

In a later section we shall attempt a closer analysis of these factors (cf. p. 139).

Fig. 6 contains further, a record of the mechanical tension after maximal stimulation of the same fibre during fatigue. The peak tension is about 40 per cent lower, but otherwise the course corresponds to the non-fatigued fibre.

A

The quotient  $\frac{A}{A+I}$  can here during reduced contraction, show a smaller change than in the non-fatigued fibre, but the deviation is not regular, and amounts to from 0—0.5 per cent of the height of compartment.

#### 4. The Cross Striation and Tension at varying Strengths of Stimulation.

In agreement with GELFAN and GERARD (1930), GELFAN (1934), ASMUSSEN (1934), and BROWN and SICHEL (1936), and in contra-distinction to STEIMAN (1937), we find no "all or none" reaction in the directly stimulated fibre. Direct stimulation of isolated curarised fibres with increasing strength of stimulation produces graded contractions propagated over the entire length of the fibre. Fig. 7 shows the extra-tension at different strengths of stimulation which varies in the ratio 1 : 3. One would expect that this considerable gradation would be reflected in a measurable degree in the quotient  $\frac{A}{A+I}$ .

At the weaker contraction, an

essentially smaller change in  $\frac{A}{A+I}$  compared with that during maximal contraction could be expected. The experiments show,

however, that the quotient  $\frac{A}{A+I}$ , within the present limits of accuracy, does not reveal real differences.

For measurements taken at the same time interval, differences of 0.3—0.5 per cent of  $A+I$  are found in the different curves, differences which do not exceed the standard deviation. Also, as can be expected, deviations in the position of the individual points with reference to the mean curve are not beyond the limit of error. Furthermore, the gradients of the individual curves were examined in the different regions, without finding a real difference, in spite of the greater measuring accuracy.

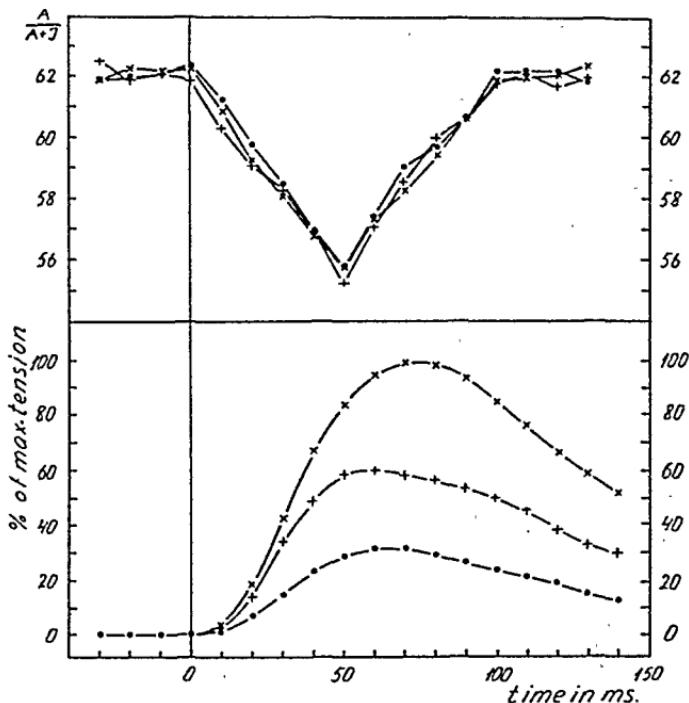


Fig. 7. Changes in cross striation and tension as function of time for isometric twitches at different strength of stimulation.

above: change in cross striation.

Ordinate:  $100 \times \frac{A}{A+I}$ .

below: mechanical tension.

Ordinate: fibre tension in per cent of the maximal extra-tension.

Abscissa: time in ms.

— · — · — approx. threshold stimulus (a).

+ — + — + —  $1.5 \times a$ ,

$\times$  —  $\times$  —  $\times$  —  $6 \times a$ .

From this may be concluded that a gradation of  $\frac{A}{A+I}$  for the elongations examined (length 100—145), if present at all, is, in any case, below 1 per cent of  $A+I$ , while the extra-tension varies simultaneously in the ratio 1 : 3.

## Discussion.

The results found — that the changes in length in the anisotropic and isotropic substance during isometric contraction up to 50 per cent of stretch, are independent of the degree of tension developed — necessitate a more detailed account of how the contraction process is reflected in tension, as well as in the changes

in structure. Even if the gradation in the contraction of the directly stimulated fibre is of no immediate importance for the physiological activity of the fibre, as the innervated fibre has an "all or none" reaction (KEITH LUCAS, 1905, ASMUSSEN, 1934), an analysis of gradation in the directly stimulated fibre may contribute towards an understanding of the contraction processes.

*What possibilities exist for the gradation of contraction in the fibre?*

- a) The number of compartments in contraction varies.
- b) The contraction is distributed equally over all compartments, but the number of contracting rods per compartment varies with the degree of stimulation.
- c) All the rods participate equally in the contraction, but the number of their active micellæ varies with the degree of contraction.
- d) All the micellæ take part in the contraction, but the number of their molecule aggregates which participate, varies with the gradation of contraction, or all the molecule aggregates are equally active during contraction, but the number of their single molecule threads, participating in the contraction, varies with the degree of contraction.
- e) All the molecules are equally active during contraction, and the grading takes place in the single molecule threads.
  - a) A grading of the number of contracting compartments corresponds to *local contractions*. These appear when applying microelectrodes and weak stimuli (GELFAN, 1930, BUCHTHAL and PÉTERFI, 1934) but, with the present technique of stimulation, the entire fibre has shown the stated characteristic changes in cross striation.

In a series of preliminary experiments, we have compared the tension developed at different strengths of stimulation, when stimulating the one end of the fibre (about 5 per cent of the total length), with a stimulation that takes place so that there is a potential gradient along the entire fibre. *Both methods of stimulation result in the same tension developed, and the gradation is practically identical.* In accordance with the changes in cross striation, this indicates that contraction is propagated over the entire fibre, and that no decrement of importance is found with the spread of locally released contractions. *We must, therefore, conclude that the intensity of the propagated wave of contraction*

*varies with the strength of the stimulus and progresses over the whole fibre without decrement.*

A combination of propagation and grading is somewhat surprising when viewed in the light of experiences from nerve physiology, as the analogy here claims that the contraction wave, after release, will either lose its intensity as "local potentials" at sub-threshold stimulation, or, with stimuli above the threshold, approach a definite limiting value. *Thus, in the muscle fibre, we have a combination of propagation and gradation* — a more detailed analysis of which mechanism will be attempted later. Furthermore, the propagation experiments have shown that the changes

in  $\frac{A}{A+I}$  are propagated without decrement over the entire length of the fibre.

In so far as investigation of the mechanical and structural properties can give information, the contraction progresses homogeneously over the fibre.

b) The next possibility, *that the number of contracted rods (micelle bundles) in the individual compartment is graded*, cannot be immediately dismissed. Such a mechanism of gradation would however involve a deformation of the basal membranes, or result in a more irregular transition between the A and I substance during weak contractions, than when at rest or when maximally contracted. Displacements in the cross striations, as they can occur during contraction at extreme degrees of stretch, are indications of an inhomogeneous distribution of contraction. But this is only an exception. There is no microscopically visible sign of inhomogeneous distribution of contractile elements in the compartment of the normal fibre.

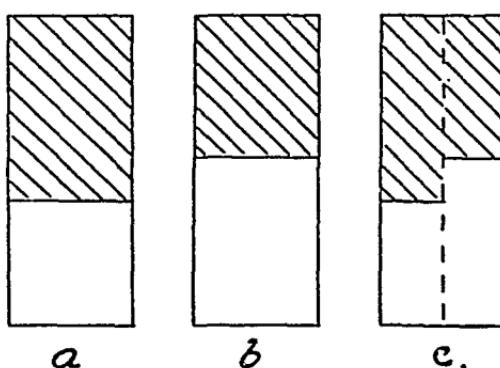
c) There is, therefore, reason to assume that all the rods participate equally in the contraction, and that grading occurs within the single rod.

With reference to d) and e), we lack, at present, means of a direct differentiation. The length of the anisotropic and the isotropic substance is determined by their equilibrium lengths, and

their stiffnesses. The fact that  $\frac{A}{A+I}$  is identical for different degrees of contraction indicates a mutual relation between the change in stiffness and the change in equilibrium length in the A and I substance of the contracting fibre. If grading took place

evenly in all the molecules, (*possibility e*), one would have to assume that A and I molecules followed the same mutual relation regarding stiffness and equilibrium length exactly compensating the changes in length. Such a dependence *may* exist, but seems rather improbable.

If, on the other hand, an "all or none" reaction in the micelle or the molecule threads (d and e), is assumed, the experimental facts can be explained in a simple manner. *Presupposing an "all or none" reaction of the micelle or molecule threads, how is the*



*Fig. 8. Diagram of a fibril within the muscle compartment (A rod + I rod)*  
a) rest, b) maximal contraction, c) submaximal contraction, where the contracting and the non-contracting substances are considered separated by means of a vertical plane.

*length of the anisotropic and isotropic substance affected by graded contractions?*

During *sub-maximal* contraction, we shall find contracted and non-contracted elements of the same length lying beside each other, equally distributed throughout the rods. During *maximal* contraction, A's part in the height of compartment is determined by the shortening of A and the change in stiffness in A and I. During *sub-maximal* contraction, the length of A and I is dependent mainly on the contracted elements; the resting elements, due to their slighter stiffness, following passively the movements of the contracted elements. In the range of stretch examined (length 100—150), the stiffness at rest is from 6—20 times less than the stiffness during contraction referring to the same fibre length (unpublished experiments with single contractions). From this point of view, we shall now try to analyse quantitatively the

influence of the resting substance upon the quotient  $\frac{A}{A+I}$  during sub-maximal contraction.

Fig. 8 a shows a compartment element (a fibril consisting of A + I rods) at rest. Fig. 8 b, a compartment element during maximal contraction, and Fig. 8 c, a compartment element in submaximal contraction, the contracted and non-contracted substances being represented as separated from each other by a vertical plane. For the one half of the diagram (8c), the resulting quotient

$\frac{A}{A+I}$  is determined by that known from maximal contraction, and for the other half by that known for the resting fibre. When the two halves are coupled together a tension arises between them, and the contracted A substance will be slightly elongated by the resting elements, while the softer resting A substance will be somewhat shortened through the contracting elements. An intrinsic tension ( $T_i$ ), is thus produced between the two halves, which is expressed by the equation:

$$T_i = \frac{A_{\text{rest}} - A_{\text{contraction}}}{\frac{1}{St_c} + \frac{1}{St_r}}, \dots \dots \dots \quad (1)$$

where  $St_c$  and  $St_r$  are stiffness referring to the change in length of the transitory regions between the A and I substance, for the contracting and resting elements respectively.

Further:

$$\frac{T_i}{St_c} = \Delta X_c \dots \dots \dots \quad (2)$$

and

$$\frac{T_i}{St_r} = \Delta X_r, \dots \dots \dots \quad (3)$$

where  $\Delta X_c$  is the deformation of the contracting substance caused by stiffness in the resting substance, and  $\Delta X_r$  the deformation of the resting substance caused by stiffness in the contracting substance.

From (2) and (3) we get:

$$\frac{\Delta X_c}{\Delta X_r} = \frac{St_r}{St_c} = G. \dots \dots \dots \quad (4)$$

For maximal contraction ( $C_{\text{max}}$ )  $\frac{St_r}{St_c}$  is termed  $\frac{1}{f}$ .

Then stiffness of the contracting substance becomes:

$$a \times \frac{C}{100} \times f \dots \dots \dots \quad (5)$$

and stiffness of the resting substance:

$$a \times \frac{C}{100}; \dots \dots \dots \quad (6)$$

where C denotes the part of the contracting elements in the fibril in per cent, and a, a proportional factor.

Thus:

$$\frac{St_r}{St_c} = \frac{100 - C}{C} \times \frac{1}{f} = G \dots \dots \dots \quad (7)$$

$\Delta X_c + \Delta X_r$  corresponds to the difference between the length of A at rest and A during contraction, and amounts to at most 6.5 per cent of A+I in the fibres investigated.

Therefore:

$$\Delta X_c + \Delta X_r = 6.5 \% \dots \dots \dots \quad (8)$$

From (8) and (4) we find:

$$\Delta X_c = \frac{6.5}{\frac{1}{1+G}} \dots \dots \dots \quad (9)$$

From these equations for G and  $\Delta X_c$ , f being valued at 10 (fibre length 130), we find  $\Delta X_c$  for different degrees of contraction (C in per cent of maximal contraction). The calculated changes in  $\frac{A}{A+I}$  caused by the shunting resting substance, expressed in per cent of the height of compartment ( $\Delta X_c$ ) corresponding to different degrees of contraction from 0—100 per cent, are represented in the following table:

Table I.

C	X <sub>c</sub>	6.5 per cent
0 (rest)		
25	1.5	»
33	1.1	»
50	0.6	»
75	0.2	»
100 (maximal contraction)	0	»

For the degrees of stretch investigated (length 100—150), where  $f$  is larger than, or equal to 10, we cannot expect to find in so far as the present considerations hold good, that gradation in tension is accompanied by measurable differences in  $\frac{A}{A+I}$ . It can

be seen from the table, that for a stimulus that initiates a contraction tension amounting to 33 per cent of the maximum,

$\frac{A}{A+I}$  deviates only 1 per cent from the quotient during maximum contraction. On the other hand, at higher stretches where the resting stiffness, on account of tension, approximates the contraction stiffness ( $f$  decreases and approaches 1) a measurable grading in

$\frac{A}{A+I}$  may be expected. To control the quantitative accuracy of these considerations, we have performed a number of experiments on fibres with higher original tensions at rest, and find — for example for a length of 170 — an appreciable difference in the change of  $\frac{A}{A+I}$  evoked by threshold and by maximal stimuli respectively.

At length 170  $\frac{\text{stiffness contraction}}{\text{stiffness rest}}$  ( $f$ ) in the mean, amounts to 2.5 (unpublished experiments). From (7) and (9), this gives a gradation in  $\frac{A}{A+I}$  ( $X_c$ ) of  $2.3 \pm 0.3$  per cent between maximal and 33 per cent contraction. Experimentally, a difference of approximately 1.5 per cent is found, while up to length 140 no

measurable difference in  $\frac{A}{A+I}$  can be detected. If there is a difference, it does not exceed 0.5 per cent. Although the difference found experimentally amounts to only  $2/3$  of that calculated, *the fact that a measurable gradation exists at high elongations, and a low, or immeasurable, at small degrees of stretch, indicates that the interpretation given is correct.* Quantitative conformity would be brought about by assuming  $f$  to be about twice its size. In this connection, it may be considered that the measured  $f$  applies to

the total fibre, and not only to  $f$  in  $\frac{A}{A+I}$ . If there were a stiffness shunting the compartment, and not involved in contraction, the

difference between measured and calculated values could be explained by assuming that the stiffness of this shunt equals approximately that of A and I at rest. We have previously discussed the rôle of the sarcolemma in the resulting stiffness of the fibre (BUCHTHAL, 1942), and it is improbable that it plays an essential part in this shunt stiffness. The present experimental material allows no deeper investigation of this problem.

Apart from the problems connected with the change of  $\frac{A}{A+I}$  in graded contractions, it is striking that the mechanical tension of the fibre can be quite appreciable, and amount to as much as 70 per cent of the peak tension, after the change in cross striation has apparently returned to its original value. This is due, either to a viscosity or a contraction remainder which is distributed over A and I in such a way, that  $\frac{A}{A+I}$  does not differ essentially from its resting value.

An explanation of the delay in the relaxation of tension by viscosity due to shunt or series viscosity respectively, can probably be excluded. Concerning the shunt viscosity over the compartment, this cannot assert itself in the *isometrically* contracted fibre, as the length of the fibre remains constant. As far as a viscosity in series is concerned, one would expect that the tension

would decrease towards zero before  $\frac{A}{A+I}$  reaches its resting value,

as this type of viscosity would tend to increase the equilibrium length of the compartment. A transport of fluid between different parts of the compartment which could explain the phenomenon is quantitatively of minor importance as has been shown by previous experiments (BUCHTHAL 1942).

We have left the assumption that the tension remaining results from an unfinished contraction process, in spite of  $\frac{A}{A+I}$  having reached its original value. The rate with which the contraction tension disappears is dependent on the start velocity of the restitution process. If we assume that the contraction process in I disappears more slowly than that in A, the latter will be stretched by the I substance still being in stronger activity (increase in stiffness in I). The course of tension in single contractions at different degrees of stretch supports this interpretation. At high

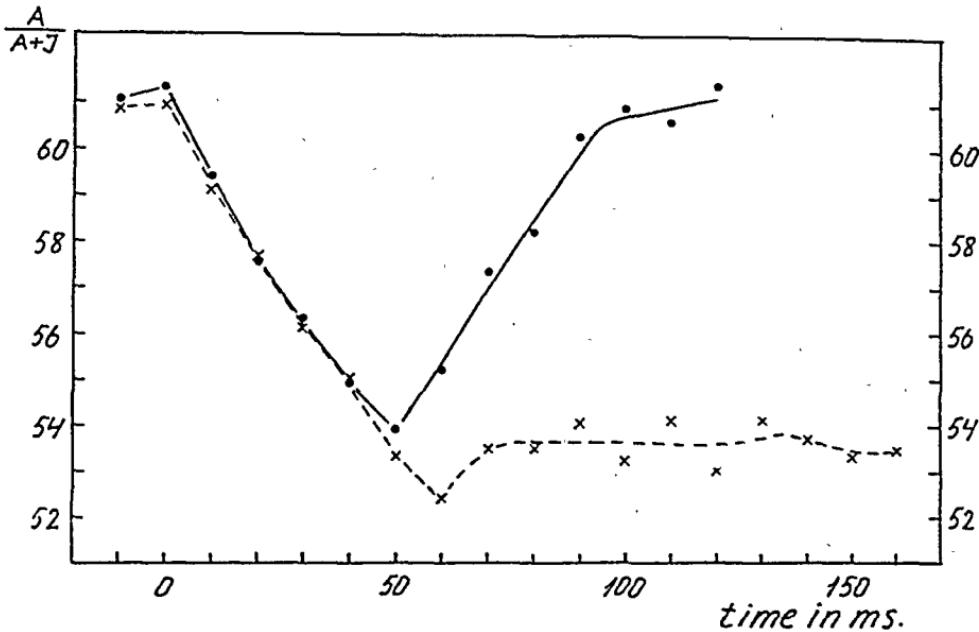


Fig. 9. Change in the cross striation as a function of time, maximal stimuli.

Ordinate:  $100 \times \frac{A}{A+I}$ .

Abscissa: time in ms.

— · — · — isometric single contractions.  
× — × — × — isometric tetanus.

elongations, the main part of tension lasts longer than in the unstretched fibre. As I's part in contraction enlarges with increasing stretch, I's influence upon the time course of contraction will increase correspondingly (cf. length-tension diagrams of A and I, BUCHTHAL 1942).

It may be of interest in this connection to mention a comparison between single contraction and the building up of the tetanic contraction (Fig. 9). In tetanic contraction, a maximum in the change of  $\frac{A}{A+I}$  is found 10 ms before the stationary value in cross striation is reached (the stationary value of tension is reached long after). This observation could indicate that the contraction in A develops 10—20 ms more quickly than the contraction in I. Therefore I reaches its maximum later, and  $\frac{A}{A+I}$  will,

in its increasing phase, exceed the stationary value. Naturally this phenomenon cannot be observed in single contraction, as a comparison with a stationary value is lacking.

Thus, 100 ms after stimulation,  $\frac{A}{A+I}$  has reached its original value, in spite of the fact that the tension — still present — implies that the contraction cannot be finished. *That cross striation has apparently reached its resting value is explained by a compensation of A's tendency to shorten through the relatively greater activity of I in the last phase of contraction.* This compensation is seldom complete, as the quotient in the last phase of the contraction can lie over or under the original value.

On the basis of the previously described molecule model (BUCHTHAL 1942), and the present considerations, we shall sum up the assumed action of the contraction mechanism in connection with grading. The simplest molecule equivalent is represented by a spiral structure with an unequal series of charges (e. g. 3) Fig. 10. By means of a local change in the electric charge of a dipole element in the molecule chain a contraction process may be released. Initiating the contraction between two opposite dipoles, a charge causing a local contraction is released, which in turn releases a charge (hereafter termed "*stimulation quantum*"), and the contraction is hereby propagated until the distance between the dipoles is so considerable, that the contraction cannot continue. One part of the molecule is now resting, the other in contraction. The progress of the charge will cease after this, and, at the first opportunity (arising from thermal movements), it will jump to a neighbouring resting chain, where the same process is repeated (Fig. 11). Fig. 11 a shows the molecule aggregate at rest, and 11 b, the molecule aggregate while the charge is on the way in the contracting molecule. The grading of contraction for the whole fiber is, therefore, determined by the energy conveyed by the stimulus, or, in other words, by the number of "*stimulation quanta*" which are propagated over the fibre. The number of stimulation quanta increases with the strength of stimulus, as does the number of contracting molecules, but *not* their degree of contraction.

The rate of propagation over the single molecule thread is likely

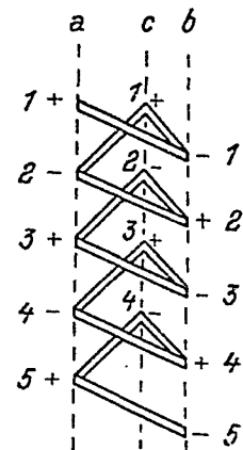


Fig. 10. Molecular chain with 3 series of charges (a, b, c).

to be very considerable. The delay is probably determined by the speed with which the stimulation quanta exchange between the single threads of the molecule aggregate, and thus the rate of propagation can become measurable.

The proportion of contracted and non-contracted substance within the individual molecule threads is determined by their degree of stretch. When this is very slight, the whole thread will contract (e. g. in a tensionless contraction); when the degree of

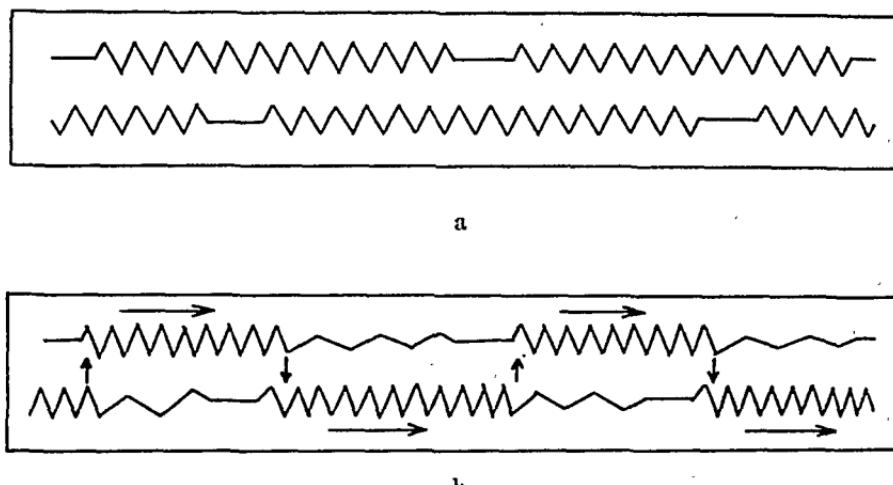


Fig. 11. Transport of a "stimulation quantum" over a molecule aggregate. a) molecule threads at rest, b) molecule threads in contraction while the charge is under way. Course of stimulation quantum marked with arrows.

stretch is moderate, the thread will contract until the distance between the resting elements is so great that the process cannot be propagated. When the elongation is extreme, the distance between the resting elements is too great for the propagation to be initiated. Thus there are two ways of regulating the degree of contraction of the fibre — 1) when the stimulus is graded, the number of contracting elements will be varied (*the resting substance shunts the contracted substance*) and 2) when the degree of stretch is varied, the proportion between the contracted and the non-contracted substance in the single elements will be varied, (*resting substance in series with contracting substance*). Additional factors, such as yielding and locking, concerning tetanic contraction are not discussed in this connection.

In this equivalent picture for the contraction mechanism, we have not considered a possible fundamental difference in structure or reaction of the anisotropic and isotropic substance.

It was shown in a previous investigation that I takes an active part in the contraction process (BUCHTHAL 1942) and has not only a "nutritive" function (CASPERSSON and THORELL 1942). Length-tension diagrams and determination of stiffness of A and I indicate that the tension developing during contraction is due to a change in the equilibrium length and stiffness in the anisotropic substance, and to an increase in stiffness in the isotropic substance. Therefore, as both substances take active part in the contraction, the simplest explanation is that the contraction molecules in the A and I substance function in a similar way, with this difference, that the molecule chains in the anisotropic substance are well orientated, while those in the I substance are distributed at random as long as the substance is not exposed to loading. This hypothesis is in contradiction to H. H. WEBER's (1934) assumptions concerning the distribution of myosin in the fibre. According to WEBER, the contractile substance is identical with Myosin, and found exclusively in the A substance. WEBER bases his supposition on quantitative extraction of myosin from muscle, and on comparative determinations of birefringence of myosin threads and muscle fibres (NOLL and WEBER 1934). In these calculations, it is assumed that the anisotropic substance constitutes 40 per cent of the height of compartment, while actually, in the living fibre (frog and mammal), it amounts to about 60 per cent. This distribution would result in a myosin deficit according to the values for the amount of myosin and double refraction assumed by WEBER. But, even if the amount of myosin found by chemical extraction implies that it is insufficient to fill the space of A as well as I, it is conceivable that the actual conditions in the fibre itself make a quantitative comparison between myosin thread and muscle fibre rather difficult. Our working hypothesis that, apart from orientation, there is no fundamental difference between the molecule threads in the two substances is in agreement with the assumptions of HÜRTHLE (1931), and W. I. SCHMIDT (1934, 1935), based on experiments with fixed materials. It is furthermore supported by different observations from experiments on living fibres:

- 1) The difference between the static elasticity moduli of A and I at rest and during contraction is very slight.
- 2) The release diagrams for the two substances under contraction are uniform, as long as the substance is above equilibrium length.

3) Yielding and locking in tetanic contraction appear in both substances.

Besides these findings, which imply a general agreement concerning the mechanical characteristics of the molecule chains, attention is drawn to the X ray structure investigations. As far as they allow comparison between myosin and muscle, the similarity in the patterns indicates that the molecules of the I substance do not deviate essentially from the A substance (myosin). A and I's different refraction for visible rays as well as the double refraction in A can also be explained by supposing a different orientation of the main axes of the molecules.

### Summary.

The mechanical reaction of the isolated cross striated muscle fibre and the time course of the structural changes are investigated by means of microcinematographing the changes in length occurring in the anisotropic and isotropic substance, and recording the mechanical tension simultaneously. A's part in the height of compartment  $\left(\frac{A}{A+I}\right)$  varies continuously between 64 per cent at rest, and 56 per cent at the apex of contraction. The apex of the single contraction is reached 50 ms after the last resting point, and after a further 50 ms  $\frac{A}{A+I}$  returns to the resting value.

The change of  $\frac{A}{A+I}$  occurs more quickly immediately after rest than during the remainder of the time course. There is a striking conformity between the decrease and increase in the change of  $\frac{A}{A+I}$  in spite of the essential difference in the simultaneously registered course of tension.

The time course of the change in  $\frac{A}{A+I}$  during contraction and the size of the change in the range of stretch investigated (length 100—145), is independent of the stretch and amounts to about 100 ms.

In spite of the fact that  $\frac{A}{A+I}$  reaches its resting value after

100 ms an essential mechanical tension is still found. This difference may be explained by assuming a different time course of the contraction and relaxation process in A and I.

In accordance with previous investigations, no "all or none" reaction is found in the directly stimulated (curarised) fibre.

The change of  $\frac{A}{A+I}$  propagates over the fibre without decrement. In experiments comparing local stimulation of the one end of the fibre with stimulation of the entire fibre, the same tension and gradation is found with both methods of stimulation. Locally released contractions are therefore propagated over the fibre without decrement. The intensity of the propagated wave of contraction is thus graded with the strength of stimulation, and proceeds over the fibre without decrement. Thus in muscle, in contradistinction to nerve, we have a combination of propagation and gradation.

Simultaneous recording of the change in cross striation and tension at varying strength of stimulation shows that there is no

measurable grading in  $\frac{A}{A+I}$  at equilibrium length and moderate degrees of stretch, whereas the mechanical tension varies in the ratio 1 : 3. To explain this apparent constancy in  $\frac{A}{A+I}$  at different degrees of contraction, one must either assume a very complicated mutual compensation between the changes in stiffness and equilibrium length in the A and I substance, or reckon with an "all or none" reaction of the minute structure elements. The latter possibility is a more simple explanation of the experimental observations. According to whether the contraction is weak or strong, a larger or smaller amount of resting submicroscopic elements shunts the contracting units.

A quantitative examination of this assumption shows that a grading of contraction cannot induce measurable changes in

$\frac{A}{A+I}$  as long as the resting tension is relatively small. Only with a high degree of stretch (length 170), can a measurable gradation be expected in  $\frac{A}{A+I}$ . The theory is confirmed by experiments. On the basis of the molecule equivalent previously described, and

the present observations, a structural interpretation of the contraction mechanism is attempted.

The authors are indebted to Dr. H. KING, National Institute for Medical Research, Hampstead, for his kindness in supplying us with purified curarinechlorid, and to Mr. E. KAISER, Engineer, for valuable advice concerning the treatment and interpretation of the material.

The present work has been supported by grants from the *Michaelsen Foundation*, and the *Nordisk Insulin Foundation*.

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## Some Inhibitory Phenomena in the Dorsal Root Reflex.

By

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Received 17 May 1943.

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Work in this laboratory on the afferent discharges, recorded with microelectrodes in intact dorsal roots (GRANIT and SKOGLUND, 1943), necessitated control of the efferent discharge in the same fibres. It was then noted that the technique used for stimulation offered certain possibilities for analysis of questions pertaining to the properties of the so-called dorsal root reflex. This technique consisted in stimulation of the afferent nerves with linear currents of variable strengths, gradient and duration (SKOGLUND, 1942).

Efferent effects in the dorsal root in response to stimulation of various afferents were first described by GOTCH and HORSLEY (1891), rediscovered by BARRON and MATTHEWS (1935), and further analyzed by TOENNIES, 1938. BARRON and MATTHEWS first believed this discharge to take place in recurrent fibres, but TOENNIES showed that it had a central reflex time of 3—4 msec., a fact incompatible with uninterrupted conduction. This has since been confirmed (BARRON, 1940, HURSH, 1940). TOENNIES found this dorsal root reflex to be present in about 35 % of the afferents but BARRON and MATTHEWS (1938 a) later showed such was the case only when the cord was cooled. If cooling was prevented the efferent discharge in the afferent roots became so small as to suggest that it hardly could be part of a normal mechanism. However, TOENNIES (1939) still claims that there is a welldefined root reflex also at normal temperatures.

Of great interest is BARRON and MATTHEWS' (1938 b) finding that the discharge occurs on the rising phase of a slow electrotonic potential in the roots which they ascribe to presynaptic terminal fibres. For this reason they believe that the discharge is a direct activation of the sensory root fibres by the electrotonic potential. This discharge is favoured when accommodation in the fibres is diminished by cooling, strychnine, or other influences (BARRON and MATTHEWS, 1938 c).

By using linearly rising stimuli of variable duration we have means of varying the form of the afferent discharge eliciting the dorsal root reflex, a method which throws some light upon certain aspects of the problem.

## Technique and Procedure.

### Stimulation.

The stimulation device, described in detail by SKOGLUND (1942), delivers linearly rising currents of strictly controllable gradient and strength through the anode circuit of a valve. The stimulator is connected so as to shift the one beam of a double cathode ray in proportion to the rate of rise of the stimulating currents, the other beam being used for simultaneous records of the discharge in the nerve through a condenser coupled amplifier (see fig. 6). The stimulus could be driven up to a certain strength at any desirable rate and be kept at plateau height for any duration desired (as in fig. 2). The stimulator could also be operated iteratively (as in fig. 4).

### Preparation.

The spinal cord of decerebrated cats was laid bare in the lumbar region. The dorsal roots, generally L 5—L 7, were sectioned on one side near the spinal ganglion and isolated up to the cord.

The cat was placed in a metal incubator, heated from below. A layer of water covering the bottom of the box maintained a warm, moist atmosphere. In addition the cord, covered with cotton wool, could be warmed by an electric radiator. The temperature of the cord was controlled by a thermometer.

The stimulating electrodes were silver-silverchloride pins, well chlorinated. As a rule they were placed on the peripheral stump of the cut root or a fascicle, generally L 7, sometimes also on the freed sciatic nerve at the level of the knee or on the saphenous. A pair of platinum hooks serving as leads to the amplifier were placed on the same root as the stimulating electrodes somewhat nearer to the cord. This arrangement was adopted in order to be able to record the input discharge. Another similar pair of electrodes, connected to a fascicle of an adjacent

root, recorded the output "reflex discharge". By a switch the amplifier could be shifted from the one pair to the other. Successful experiments were carried out with 13 cats.

## Results.

### Observations on some known Properties of the "Reflex" Discharge.

In fig. 1 the form of the stimulus is shown in the records. In *a* and *c* the time marker interrupts the stimulus beam every msec., in *b* and *d* every 2 msec. In record *a* L 7 has been stimulated at a distance of 25 mm. from the cord and the effect appears in the record from a point just at the cord after 1 msec. The root reflex to a slightly stronger stimulus, is pictured in *b*, recorded from L 6. The uncorrected latent period is 5 msec. leaving just below 4 msec. for the central processes in agreement with the results of other workers. Record *c* illustrates the reflex in L 6 to stimulation of the sciatic nerve. The increased conduction distance has increased the latent period to about 6 msec.

In record *d* the dorsal root reflex, picked up as in *b*, is preceded by a rapid negative potential of extremely short latent period. This phenomenon occurs when the stimulating electrodes are so near the spinal cord that they have a chance of stimulating the latter directly (cf. BARRON and MATTHEWS, 1938 b). Thus this potential remains after pinching of the "input" root between the stimulating electrodes and the spinal cord, preventing all impulses to reach the latter so that no dorsal root reflexes are set up.

In these experiments with instantaneously rising currents it was also confirmed that the temperature was an essential factor in determining the size of the reflex discharge. Just after the dura

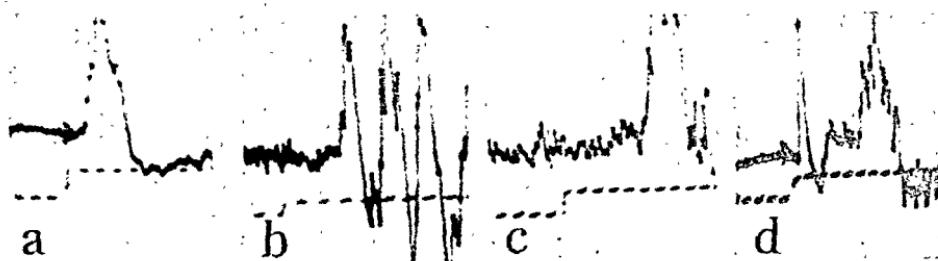


Fig. 1. *a* afferent, *b*, *c* and *d* efferent discharges in dorsal roots caused by stimulation with instantaneously rising currents. Lower beam in this and the following figures marks stimulus form and time. Full description in text.

had been opened in the beginning of the experiment, very few fibres became "reflexly" activated, despite of the large input volley. Sometimes no effect whatever was obtained. Somewhat later dorsal root reflexes appeared with stimuli slightly above rheobasic strength for the input discharge. This was noted in our first experiments. When in the later experiments the temperature was controlled the increased dorsal root reflex was found to be accompanied by a sinking of the temperature by  $3^{\circ}$ — $4^{\circ}$ . The radiator was then introduced and by warming up the spinal cord to  $38^{\circ}$ — $39^{\circ}$  the process was found to be reversible. This was the only factor found to determine the size of the dorsal root reflex in a reversible manner. Decerebrated animals were given dial intraperitoneally in successively increasing doses. Yet the dorsal root reflex was found to be uninfluenced until respiratory disturbances occurred.

There was a regularly noted parallelism between the size of the dorsal root reflexes and that of the ipsilateral flexion reflex at different temperatures. In a couple of experiments this was also confirmed with the ordinary reflex discharge as recorded from the ventral root (cf. GRUNDFEST (1941) who made similar observations on the flexion reflex in relation to cooling of the spinal cord).

#### Blocking of the Prolonged "Reflex" Discharge Caused by Stimulation with Constant Currents.

Continuous currents set up prolonged discharges in mammalian sensory nerves (SKOGLUND, 1942). It is of interest to see how such discharges are modified when they reappear in the reflex output. In fig. 2 *a* the afferent *input* from L 7 is recorded in response to a continuous stimulus of four times rheobasic strength. After one sec. (right) there is still a considerable slowly diminishing discharge left. *The efferent output* of an afferent in the same experiment is shown as record *b*. There is some spontaneous activity in the base-line. Nevertheless it is clear that the heavy input bombardment of impulses has a negligible effect after about 0.75 sec. This is at about  $37^{\circ}$  of the spinal cord. But in record *c*, where in the same experiment the spinal cord has been cooled down to  $33^{\circ}$ , there is an impressive discharge still after 1 sec. of stimulation. Actually this afferent output in the dorsal root lasted for 2.25 sec.

The gradually developing block in the input-output transition of the dorsal root reflex is apparently conditioned by the incoming

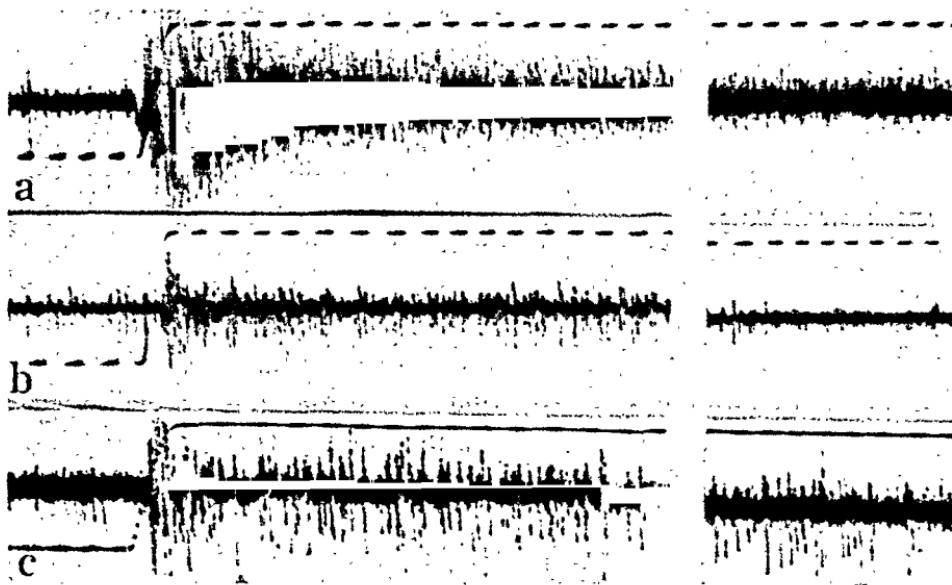


Fig. 2. a afferent, b and c efferent discharges in dorsal roots caused by stimulation with constant currents. Full description in text.

impulses themselves. But in addition the blocking effect, cutting down the discharge, depends upon the temperature of the spinal cord, being well developed at normal temperatures and far less noticeable in cooled preparations.

#### Inhibition of Intermittent "Reflex" Discharges Caused by Iterated Single Shocks of Variable Frequency.

The above experiments suggested that a state of inhibition is built up by the successive impulses of a discharge. Whether this is so may be tested by sending dispersed input discharges of constant size at constant intervals and studying the size and duration of the output discharge at different temperatures.

In fig. 3 *a* the input volleys to L 7 have been caused by brief excitatory shocks at intervals of 50 msec. A sweep synchronized with the initiation of the stimulus has been used. The form of the latter is covered by the action potentials of the input discharge in *a*, but in *b*, where the corresponding afferent root reflexes have been recorded, the stimulus (see beam marking it) can be seen preceding the discharge, owing to the longer latent period of the dorsal root reflex. The input volleys, iterated in *a*, are seen to be of constant size, but the reflex discharge is well developed only

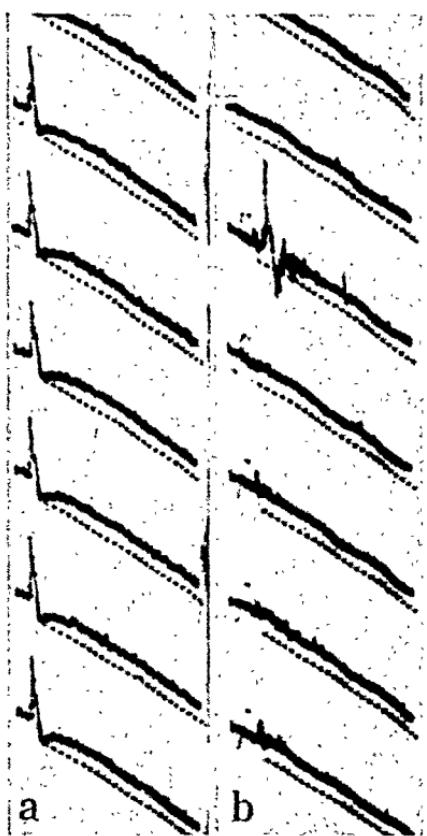


Fig. 3. a afferent, b corresponding efferent discharges in dorsal roots caused by repeated stimulation with short current pulses at a frequency of 20/sec. (see text).

cord the reflex discharge diminished very slowly on repetition showing that the inhibitory state rose at a very slow rate.

This difference between the normal and the cooled spinal cord is typical. In another experiment it was still more pronounced. In the beginning of that experiment inhibition was instantaneous at a frequency of 20 per sec; when the cord had been cooled, it lasted about 25 sec. before the impulses had been completely inhibited. For a spinal cord in any given state an increase

in response to the first stimulus of the series. In the others it is very small. A similar inhibition by repetition has been noted by TOENNIES (1938). In this particular case the inhibitory state developed rapidly.

Fig. 4, however, is from a case in which inhibition developed slowly. Stimulus form and frequency were as in fig. 3. That the input volleys were constant was checked by observation on the screen. Only the reflex effect was recorded in this experiment; however, on a standing film so that a few sweeps became superimposed. Record *a* shows the large instantaneous effect of the first stimuli throwing the cathode ray out of the picture. Record *b* is an exposure after 3 seconds, corresponding to the 60th stimulus, and record *c* after 6 sec. to the 120th stimulus. In this experiment with a cooled spinal

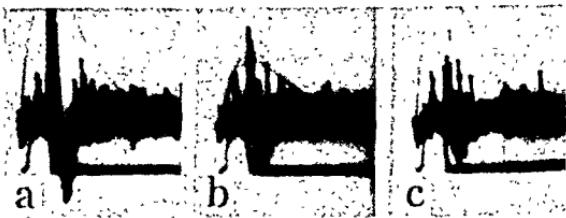


Fig. 4. Efferent dorsal root discharges caused by repeated stimulation with short current pulses. Superimposed pictures of several sweeps in each record, a at beginning of stimulation, b after 3 sec., c after 6 sec. (see text).

in the stimulus frequency led to an increase in the rate of development of the inhibitory block. An example is shown in fig. 5 in which the duration of stimulation necessary for complete inhibition is plotted on the abscissae against stimulus frequency as ordinates.

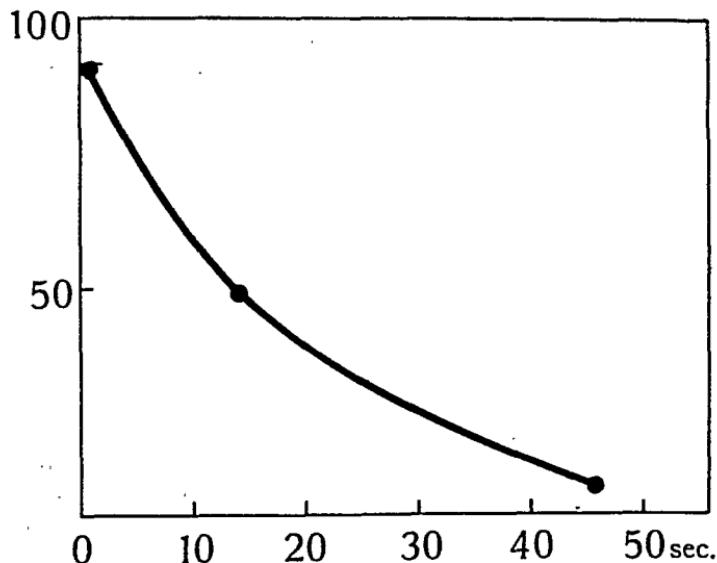


Fig. 5. Ordinates: stimulus frequency. Abscissae: duration of stimulation necessary for complete inhibition (see text).

#### Variations in the "Reflex" Discharge Caused by Variations in the Rate of Rise of Linear Stimuli.

A glance at fig. 6, records *a—d*, will show the principle of the experiment, in these records by illustrating the input discharge in the afferent root in response to linear currents of different rates of rise. The instantaneously rising stimulus at a strength of  $28 \mu A$  in *a* caused a large synchronous volley. When in *b* the same current rises more slowly the discharge diminishes and becomes less synchronized owing to accommodation. In *c*, a still more slowly rising current has been driven up to greater strength ( $46 \mu A$ ) leading to a repetitive discharge, more marked in *d*, where at the same rate of rise the stimulus has been still further increased in strength ( $60 \mu A$ ). It has been shown by GRANIT and SKOGLUND (1943) that suprathreshold stimuli actually elicit repetitive firing in single sensory fibres.

Let us now see how these different input discharges are transformed in the "reflex output", shown in records *e—g*. In *e* and *f*,

corresponding to *a* and *b*, there were well-marked output discharges. However, the input discharge of *c* failed to give an output effect. It was necessary to drive the stimulating current to greater strength, as in *d*, in order to be able to record the small discharge

of *g*. This was recorded with somewhat lesser amplification of the stimulus beam of the cathode ray, as also shown by the compressed time marking of the latter.

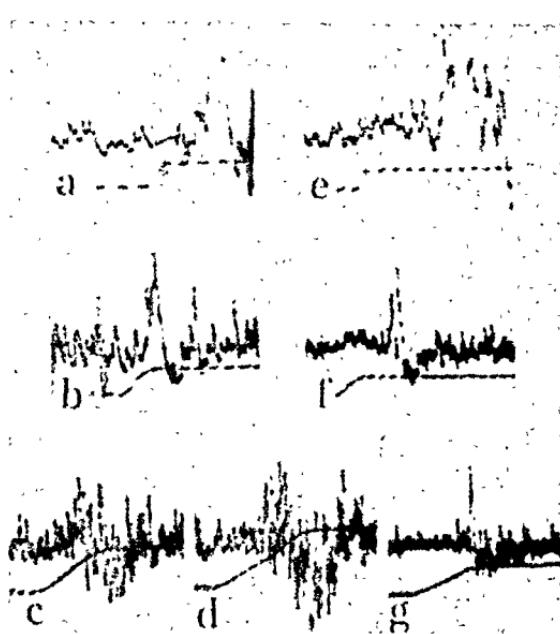


Fig. 6. *a*, *b*, *c*, and *d* afferent discharges, *e*, *f*, and *g* corresponding efferent discharges in dorsal roots caused by stimulation with linearly rising currents. Time in 2 msec. (see text).

so. In favour of this explanation are the experiments by FESSARD and MATTHEWS (1939) on frogs in which they showed that successive afferent impulses in a single fibre caused an increase in magnitude and duration of the electrotonic potential of the same root in proportion to the frequency and duration of the input discharge. Unfortunately we have not been able to record the slow electrotonic potential in this work since the slowly rising currents require special compensatory devices for an elimination of the artefact, not available at the moment.

But our experiments offer another interesting possibility for testing these assumptions. This is to compare the effects of slowly rising currents with normal and cooled spinal cords presuming the spinal cord to be affected by cooling in the direction of decreased accommodation, as we have every reason to do (cf. introduction.) It can easily be shown that the input discharges, set up by the slowly rising currents, pass a cooled spinal cord far more easily

How are these facts to be understood? It is tempting to imagine that the input discharge of record *c* sets up in the centre an electrotonic potential which arises too slowly and possibly also is too small to cope with accommodation in the central terminals. The repetitive discharge of record *g* will have enough density to do

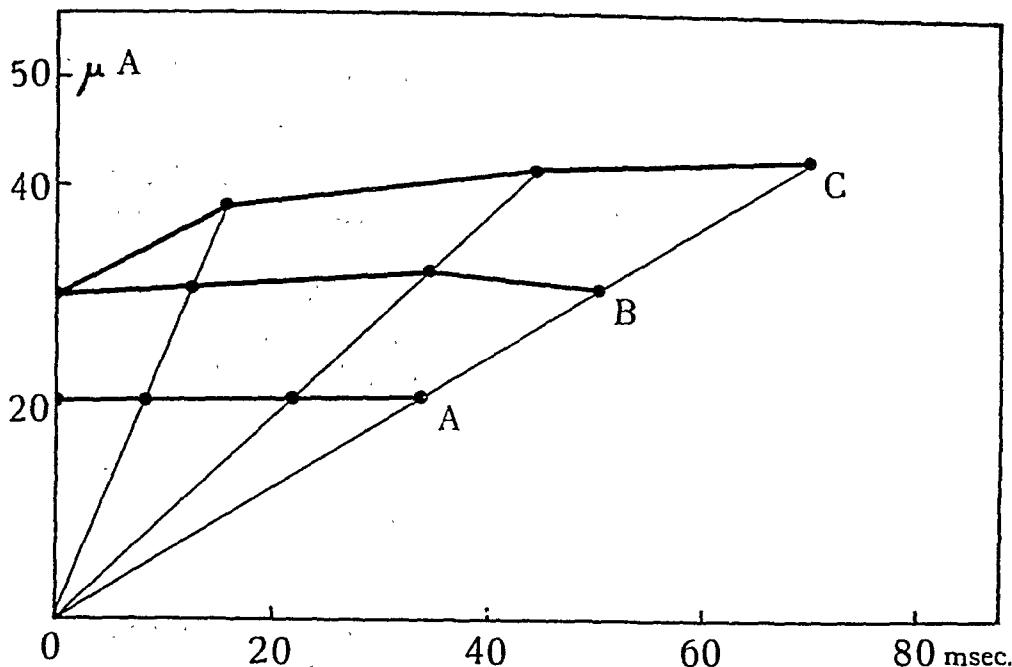


Fig. 7. Ordinates: absolute current strength in microamp. Abscissae: rising times in msec. The thin lines from origo represent the rising stimuli. Full description in text.

than a normal one. The outcome of a quantitative experiment is shown in fig. 7. Ordinates are stimulus strengths and abscissae stimulus duration. The lines from origo show the linearly rising currents inserted in the diagram. The dots connected by the line A show the threshold for the afferent input discharges in terms of stimulus strengths at the moment when the first impulse appears, in other words, the accommodation curve of the afferent. It has the typical flat run of skin afferents (SKOGLUND, 1942, GRANIT and SKOGLUND, 1943).

Similarly the line C illustrates the thresholds for the dorsal root reflex at normal temperature. Its steeper gradient signifies that the decreased rate of rise of the current has had to be compensated for by increased strength. But as soon as the cord has been cooled down, and despite of the fact that the threshold to the instantaneously rising currents is unaltered, the curve B, illustrating this case, approaches the form of A signifying that smaller current gradients do not now have to be compensated for by as large increases in strength as in C. The view that in the cooled preparation a smaller electrotonic potential of lesser gradient

suffices to overcome the diminished accommodation has thus been supported by this experiment.

### Discussion.

Our results show that the transmission of a drawn-out, non-synchronous input discharge (cf. the preceding paragraph) is impeded at normal temperatures, and that at such temperatures an inhibitory block is generated by the discharge itself. From this can be concluded that under normal conditions there must be a considerable safety margin prohibiting the occurrence of the so-called dorsal root reflex. The observation by BARRON and MATTHEWS (1938 c) that natural stimuli caused by a sharp tap on the foot elicited a reflex response whilst steady pressure failed to do so, can be explained along the same lines.

From the point of view of general properties of nervous tissue it is of interest to note that in peripheral nerve postexcitatory depressive states are better marked in fibres of good accommodation (SKOGLUND, unpublished observations), a fact, which together with the results of the last section suggests that the effect of temperature may influence a process in the spinal cord comparable with accommodation in peripheral nerve.

The question as to whether the dorsal root reflex differs from other reflexes cannot be regarded as solved, but the general similarity of behaviour, noted in the flexor reflex suggests similar determinants in both cases. Onto these factors, as well as onto their relation to the general problem of accommodation in centres this work has directed attention.

### Summary.

The efferent "reflex" output in the dorsal root L 6 in response to afferent discharges from L 7 (the so-called dorsal root reflex) has been studied in decerebrate cats with the aid of electrical stimuli of variable form. Special attention has been paid to the effect of temperature.

Prolonged afferent discharges, set up by constant currents, cause prolonged dorsal root reflexes. Increased duration of the afferent discharge is accompanied by an inhibitory block, far more pronounced at normal temperature than at low temperature of the spinal cord.

Intermittent afferent stimuli, set up by brief iterated shocks, elicit intermittent dorsal root "reflex" discharges which successively diminish in size. This depression increases with stimulus frequency and for any frequency is far more pronounced at normal temperature than at low temperature of the spinal cord.

Afferent discharges set up by linearly rising currents of steep gradient, elicit well marked "reflex" discharges. But slowly rising currents, which still give well marked impulses in the input root, fail to cause the reflex response. For this to occur the linearly rising stimuli must be increased in strength so as to cause prolonged repetitive discharges. The necessary increase in current strength is greater at high than at low temperature of the spinal cord.

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## Humoral or Nervous Control of Respiration during Muscular Work?

By

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Received 19 May 1943.

KROGH and LINDHARD (1913) concluded from their experiments that the abrupt rise in pulmonary ventilation at the beginning of work is due to a suddenly increased excitability of the respiratory centre, brought about by irradiation of impulses from the motor cortex. During the steady state of work they assumed that the pulmonary ventilation was governed by the ordinary automatic chemical regulation. NIELSEN (1936), however, showed that in the steady state of work the intensity of the chemical stimuli (arterial  $pCO_2$  or  $C_H$ ) is not increased, and furthermore demonstrated that the increased pulmonary ventilation is due to an increased excitability of the respiratory centre towards  $CO_2$ . The change in excitability might be either of cerebral or of reflex origin or it might be caused by some substance produced in the working muscles (e. g. the "hyperpnein" of Y. HENDERSON) and acting either on the carotid and aortic bodies or directly on the respiratory centre.

The purpose of this paper is to investigate if the increase in excitability of the respiratory centre during exercise can be ascribed to such substances produced in the working muscles or if it may be of nervous origin. For this purpose a series of work experiments on KROGH's bicycle ergometer was carried out during which the circulation to the lower extremities could be cut off by means of pneumatic cuffs, inflated to a pressure of 250—300 mm Hg. In this way it was possible to make sure that no substance formed in the muscles distal to the inflated cuffs could enter the main circulation, whereas the nervous connections were unaffected. Under these circumstances work could be continued with unchanged intensity for several minutes and the ventilation compared with the normal ventilation. In addition to the work ex-

periments series of supplementary experiments were performed, during which the circulation to the legs was cut off in the resting condition.

*Methods:* Metabolism and pulmonary ventilation were determined by the Douglas bag method. The frequency of respiration was registered by a Marey tambour in connection with the respiration valve. Alveolar ventilation and alveolar  $pCO_2$  were calculated from the obtained data, the dead space of the subjects having been previously determined.

The experiments were carried out on four male subjects R. J. (25 years, 172 cm, 60 kgm), M. M. (20 years, 170 cm, 62 kgm), E. A. (32 years, 172 cm, 70 kgm) and M. N. (36 years, 188 cm, 80 kgm). All determinations were made in the steady state after about 15 minutes of continuous work. The determinations with the circulation to the lower extremities cut off were made immediately after the determinations in the normal condition.

*Results:* The main experiments were performed on subject E. A. who was well trained and from earlier experiments well accustomed to the special procedures necessary for these experiments. In table I the data from the experiments on E. A. with different intensities of work are given together with the results on R. J. and M. M. with the intensity of 360 mkg/min. A more detailed analysis of the results on E. A. with the intensity of 360 mkg/min is given in tables 2 a and 2 b.

Table I shows the oxygen intakes and the ventilations during work on E. A. varying between 180 mkg/min and 900 mkg/min. The time during which work could be continued with the circulation to the legs blocked varied between 5 minutes for the work intensity of 180 mkg/min and somewhat less than 1 minute for the heaviest work. It will be seen that the oxygen intake in the experiments with the circulation to the lower extremities cut off has decreased from 22 to 49 pCt compared with the oxygen intake in the normal experiments. This corresponds to a decrease in the oxygen consumption of the working muscles of from 30 to 58 pCt. The reason why the percentage decrease in oxygen intake is so much smaller with the lighter work is presumably that the oxygen consumption corresponding to the stabilizing work here is relatively greater. In spite of the considerable decrease in oxygen intake the ventilation is practically unchanged. The experiments with the two other subjects R. J. and M. M. who were less accustomed to the experimental procedures than E. A., show in the main the same results: In both subjects the ventilation is a little increased in the experiments with the legs "cut off" in spite of the con-

siderable decrease in oxygen intake. In subject R. J. the ventilation was slightly increasing and in subject M. M. slightly decreasing during the three minutes of occlusion.

Table 1.

Subj.	Rate of work mkg/min.	Num-ber of exp.	I Normal condition		II Circul. to legs cut off		Decrease or increase of II in pCt. of I	
			O <sub>2</sub> -intake l/min.	Vent. (37°) l/min.	O <sub>2</sub> -intake l/min.	Vent. (37°) l/min.	O <sub>2</sub> -intake pCt.	Vent. (37°) pCt.
E. A.	180	4	0.735	15.1	0.575	15.7	-22	+ 4
	360	22	1.12	21.5	0.78	21.3	-30	- 1
	540	2	1.44	29.1	0.74	28.1	-49	- 3
	720	2	1.78	38.2	1.08	37.4	-39	- 2
	900	2	2.08	46.3	1.09	47.5	-48	+ 3
R. J.	360	6	1.06	24.3	1. min: 0.82	25.5	-23	+ 5
		5	—	—	2. → 0.72	26.0	-32	+ 7
		11	—	—	3. → 0.78	27.8	-26	+14
M. M.	360	4	1.09	27.4	1. → 0.70	30.8	-36	+12
		4	—	—	2. → 0.73	29.5	-33	+ 8
		4	—	—	3. → 0.84	28.5	-23	+ 4

Tables 2a and 2b show a more detailed analysis of the experiments on E. A. with the work intensity of 360 mkg/min. Table 2a contains the normal experiments and table 2b the experiments with the lower extremities "cut off". The figures in table 2a are single determinations from different experimental days. In the experiments presented in table 2b, the circulation to the legs was cut off for 2.5 min., during which time usually two determinations covering the whole experimental period were made. (1st and 2nd det. of table 2b.) The tables show, that the day-to-day variations in the pulmonary ventilation are very small, and that, in spite of the considerable difference in oxygen intake, the ventilation is unchanged in the two series of experiments. The ventilation per liter O<sub>2</sub>-consumption is consequently increased, whereas the alveolar pCO<sub>2</sub> is diminished. The ventilation is slightly decreasing during the period of occlusion from an average of 22.0 l/min. in the first determinations to 20.7 l/min in the second determinations, and so is the alveolar pCO<sub>2</sub> (from 39.1 mm Hg to 38.2 mm Hg). The depth and frequency of respiration are very nearly the same in the two conditions.

Table 2 a.

*Normal experiments.*

Subj. E. A. 360 mkg/min.

	Vent. (37° etc) l/min.	O <sub>2</sub> - intake l/min.	Vent. 37° per 1 O <sub>2</sub>	Alv. pCO <sub>2</sub> mmHg	Resp. fre- quency	Resp. depth l
	22.7	—	—	—	11.9	1.91
	22.4	1.16	19.3	42.5	12.5	1.79
	20.7	1.12	18.5	43.1	11.3	1.83
	22.2	1.15	19.3	41.8	12.3	1.80
	21.3	1.08	19.7	43.0	12.0	1.78
	22.5	1.12	20.1	41.2	12.3	1.83
	21.8	1.17	18.6	42.0	12.3	1.77
	20.8	1.07	19.4	42.0	12.8	1.63
	20.6	1.11	18.6	42.4	12.1	1.70
	21.2	1.12	18.9	41.6	12.4	1.71
	20.4	1.11	18.4	42.1	12.4	1.65
Average:	21.5	1.12	19.1	42.2	12.2	1.76
	± 0.3	± 0.01				

Table 2 b.

*Experiments with circulation to lower extremities cut off.*

Subj. E. A. 360 mkg/min.

	Vent. (37° etc) l/min.	O <sub>2</sub> - intake l/min.	Vent. 37° per 1 O <sub>2</sub>	Alv. pCO <sub>2</sub> mmHg	Resp. fre- quency	Resp. depth l
1st determ. . . . .	23.1	0.86	26.9	(42.4)	12.7	1.82
2nd   > . . . . .	21.2	0.79	26.8	38.7	13.4	1.58
1st   > . . . . .	22.9	0.85	26.9	39.7	13.2	1.73
2nd   > . . . . .	21.8	0.83	26.3	38.4	11.6	1.88
1st   > . . . . .	22.2	0.83	26.8	39.0	12.3	1.81
2nd   > . . . . .	18.9	0.79	23.4	39.3	9.7	1.95
1st   > . . . . .	19.3	0.69	28.0	38.8	9.9	1.95
2nd   > . . . . .	21.5	0.81	26.6	37.4	10.1	2.13
1st   > . . . . .	22.5	0.68	31.4	38.8	12.5	1.80
2nd   > . . . . .	20.2	0.71	28.5	37.3	10.7	1.89
1st   > . . . . .	20.5	0.73	28.1	37.4	10.8	1.90
Average:	21.3	0.78	27.2	38.5	11.5	1.86
	± 0.4	± 0.02				

Table 3.  
*Experiments at rest.*

	Number of experi- ments	Vent. 37° l/min.	O <sub>2</sub> - intake cc/min.	CO <sub>2</sub> - output cc/min.	Vent. 37° per 1 O <sub>2</sub>	Vent. 37° per 1 CO <sub>2</sub>	Alv. Vent. 37° l/min.	Alv. pCO <sub>2</sub> mmHg
E. A. normal exp. . . .	9	5.66 ± 0.06	256 ± 2.1	202 ± 2.1	22.08	28.00	4.50	39.3 ± 0.8
E. A. circul. to legs cut off . . . .	6	5.25 ± 0.09	237 ± 2.1	190 ± 2.8	22.12	27.60	4.18	40.1 ± 0.3
M. M. normal exp. . . .	9	6.93 ± 0.12	254 ± 1.2	209 ± 1.8	27.23	33.10	4.70	39.0 ± 0.5
M. M. circul. to legs cut off . . . .	12	6.26 ± 0.05	231 ± 1.4	189 ± 0.8	27.08	33.10	3.98	41.3 ± 0.4
		(-7.8 %)	(-7.4 %)	(-6.0 %)	(+ 0.1 %)	(-1.4 %)	(-7.1 %)	—
		(-9.7 %)	(-9.1 %)	(-9.6 %)	(-0.5 %)	(0 %)	(-15.3 %)	—

In table 3 the results from experiments in which the circulation to the legs was cut off in the resting condition are given. In contradistinction to the work experiments the pulmonary ventilation is here diminished in the same proportion as the metabolism, so that the ventilation per liter O<sub>2</sub> and per liter CO<sub>2</sub> is practically unchanged. The same is the case with the alveolar pCO<sub>2</sub> in subj. E. A., whereas in subject M. N. the alveolar pCO<sub>2</sub> is a little increased, due to the fact that the alveolar ventilation in this subject has decreased more than corresponding to the decrease in CO<sub>2</sub>-output.

*Discussion.* Already GEPPERT and ZUNTZ (1888) pointed out, that the increase in pulmonary ventilation during muscular work could be caused either by nervous impulses to the respiratory centre or by changes in the composition of the blood irrigating it. By experiments on dogs and rabbits in which the spinal cord was transsected at the level of the 12. thoracal segment, these authors found an increase in the pulmonary ventilation, when the hind limbs of the animals were electrically tetanized. As the arterial CO<sub>2</sub>-tension was not increased they concluded that the increase in ventilation was brought about by some unknown substance in the blood, produced in the working muscles.

In more recent years it was commonly assumed, that the  $pCO_2$  or  $C_H$  of the arterial blood was the factor governing ventilation, both in rest and in the steady state of muscular work (HALDANE, WINTERSTEIN, KROGH and LINDHARD). Later experiments (NIELSEN 1936) showed, however, that neither the  $pCO_2$  nor the  $C_H$  of the arterial blood were significantly changed in the steady state of work, but that the threshold of the respiratory centre towards  $pCO_2$  was so much lowered, that this alone could account for the increased ventilation. The question, whether this lowering of the threshold of the respiratory centre was due to humoral or to nervous influences remained open, however,

The assumption, that the increase in ventilation during muscular work is brought about by some unknown substance produced in the working muscles and passed to the respiratory centre by the blood has been advocated by Y. HENDERSON (1920 and 1938) and has quite recently been revived by some german physiologists (Koch, BENZINGER a. o. 1940). All these authors base their assumption essentially on the results of GEPPERT and ZUNTZ referred to above. As it had been objected against the experiments of GEPPERT and ZUNTZ (NIELSEN 1936) that only the exspiratory  $CO_2$  was used as measure for the arterial  $pCO_2$ , KRAMER and GAUER (1941) repeated their experiments with an improved technique, allowing a continuous determination of the alveolar  $pCO_2$ . Their results confirmed the findings of GEPPERT and ZUNTZ, that at least in the experiments on the rabbits the arterial  $pCO_2$  was not increased during electrically induced work of the hind limbs of the animals. Simultaneous with an increase in ventilation during the period of stimulation KRAMER and GAUER, however, found a decrease in arterial blood pressure, which was linearly correlated to the increase in ventilation. They concluded from these experiments, that the increase in ventilation in their experiments, and probably also in those of GEPPERT and ZUNTZ, was effected through reflexes to the respiratory centre from the pressosensitive zones of the carotid sinus and the aortic arch (HEYMANS and BOUCKAERT 1930). The assumption of the stimulation of the respiratory centre through an unknown substance produced in the working muscles thus proved unnecessary.

If the increase in pulmonary ventilation during muscular work were due to substances produced in the muscles, the amount of such substances must be proportional to the intensity of the work, as the close correlation between ventilation and metabolism otherwise could not be explained.

In the experiments here presented an occlusion of the circulation to a considerable part of the active muscles has been made, so that a corresponding amount of the hypothetical substance was prevented from reaching the respiratory centre. Consequently, one should expect to find a decrease in ventilation, which should be proportional to the decrease in  $O_2$ -intake. The experiments showed, however, that the ventilation during the period of occlusion remained almost at the normal level. It is therefore concluded, that the increase in excitability of the respiratory centre during light and moderate muscular work is not brought about by substances produced in the working muscles, but must be of nervous origin.

This conclusion is only justifiable, provided the ventilation is not otherwise affected through the experimental procedure.

As factors, which might disturb the experiments by affecting the ventilation, psychic influences and ischemic pains, which are increasing throughout the experiments must be mentioned. As a matter of fact experiments on subjects, who are not accustomed to this special procedure are easily disturbed by such influences, which, however, after a certain period of practising can be nearly eliminated. Further it was noticed, that unskilled subjects had a tendency to use new muscles, less fit for the work, during the period of occlusion, so that only relatively small and irregular decreases in oxygen intake were obtained. The results presented in tables 1, 2a and 2b are obtained from well practised subjects, and especially subject E. A. had been much trained in this experimental technique, which is seen from the fact, that the day-to-day variations in the ventilations during occlusion are very small (table 2b). A comparison between the first and the second determinations of the ventilations (average 22.0 l/min, respectively 20.7 l/min) in table 2b shows further, that the effect of the increasing ischemic pain is negligible in E. A. The other two subjects never complained of ischemic pains.

At the moment when the cuffs are inflated, the arterial blood pressure is suddenly increased, but in the course of a few seconds it returns to a level which is only slightly higher than the value in the normal steady state of work. This change in blood pressure cannot explain the relatively high value of the ventilation during occlusion. If anything, it should affect the ventilation in the opposite direction.

The question might be raised, whether the period of occlusion is long enough to allow the ventilation to adjust itself to the

new condition. In order to elucidate this problem experiments were performed, in which the intensity of work (360 mkg/min) suddenly was diminished to such a rate (about 180 mkg/min), that the oxygen intake was the same as during the period of occlusion. These experiments showed that already after 1 minute the ventilation was practically adjusted to the new level. Consequently the time of occlusion in our experiments must have been sufficient for the purpose.

Whereas the experiments with occlusion of the circulation to the legs during work showed, that the ventilation under these circumstances was not correlated to the rate of metabolism, but must be governed by nervous impulses, the experiments in table 3 show, that when no work is performed during the period of occlusion, the ventilation is closely correlated to the metabolism. This can be seen from the fact, that the decrease in oxygen intake and in ventilation are of practically the same magnitude.

### Summary.

The pulmonary ventilation and the oxygen intake during light to moderate work on the bicycle ergometer were determined under normal conditions and in experiments in which the circulation to the lower extremities was interrupted by means of pneumatic cuffs. The experiments showed, that although the oxygen intake could by this procedure be diminished by 20—50 pCt., the ventilation remained almost unchanged.

From these experiments it is concluded, that the increase in the excitability of the respiratory centre towards CO<sub>2</sub>, which occurs during work cannot be ascribed to substances produced in the working muscles, but must be of nervous origin.

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## Cortical or Reflex Control of Respiration during Muscular Work?

By

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Received 2 July 1943.

In earlier papers (NIELSEN 1936) it has been shown that the chemical stimulus of the respiration (arterial  $pCO_2$  or  $C_H$ ) is not increased during muscular work, but that the increase in pulmonary ventilation during work can be explained quantitatively by an increase in the excitability (lowering of the threshold value) of the respiratory centre towards  $CO_2$ . It has been shown further (ASMUSSEN, CHRISTENSEN and NIELSEN 1943) that the increase in excitability of the respiratory centre during exercise is not due to humoral influences i.e. to substances, produced in the working muscles and transported to the centre by the blood stream. It must therefore be supposed to be of nervous origin and to be possible only in two ways: viz. either is the increase in excitability of the centre brought about by irradiation of the cortical impulses to the muscles, in the same way as KROGH and LINDHARD (1913) explained the abrupt rise in ventilation at the onset of work — or it is due to reflex impulses from the working muscles. In both cases it is possible to explain the existing linear relation between the ventilation and the intensity of work, as the strength of both the cortical innervation and of the reflex impulses, which may be caused by mechanical changes in the muscles, must be closely correlated to the work intensity.

In order to investigate, whether the impulses influencing the respiratory centre are of cortical or of reflex origin, we have studied

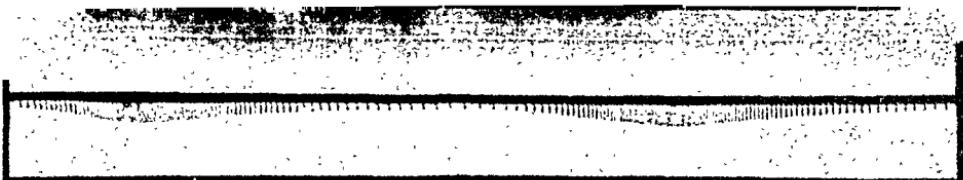


Fig. 1. Example of oscillographic recordings of impulses, set up by the "Myotensor".

the respiration in voluntary work and in electrically induced work, in which the cortical innervation was excluded and replaced by the apparatus for electrical stimulation.

### Methods.

As stimulating apparatus we used the "Myotensor"<sup>1</sup> by means of which it is possible to a high degree to imitate the natural voluntary muscular contractions; the "Myotensor" can be set automatically, as shown in fig. 1, to give waves of impulses, the frequency of which can be varied. Inside the individual waves the frequency and strength of the single impulses can be set to increase (contraction) and decrease (relaxation) from an adjustable basal frequency and strength of impulses. The time for contraction and relaxation can be varied at will. Further the duration of the single impulses can be varied corresponding to the chronaxy of the muscles.

The arrangement for the work experiments is presented in fig. 2. The working machine consists of an angular lever equipped with pedals to which the feet were fixed. The lever moved on a horizontal axis, and the load of work could be varied by means of a spring. The indifferent electrode was placed on the back of the subject, the different electrodes ventrally on the thighs and dorsally on the calves. The work consisted in a rhythmic stretching of the lower extremities. The strength and frequency of the stretching could be varied within wide limits by means of the "Myotensor". The movements of the long arm of the lever were registered on a drum to control the constancy of work. With some experience it was possible to adjust the "Myotensor" so that the electrically induced work could not be distinguished from normal voluntary work performed on the same working machine. There were no sensations from the skin covered by the electrodes, and the subject was sitting passive while the legs were working.

After a preliminary period of from 10 to 15 minutes of work the metabolism and ventilation were determined by the Douglas bag method. The alveolar  $\text{pCO}_2$  then could be calculated, the dead space being previously determined.

<sup>1</sup> Manufactured by Dansk Industrisyndikat. Compagnie Madsen A/S Copenhagen.

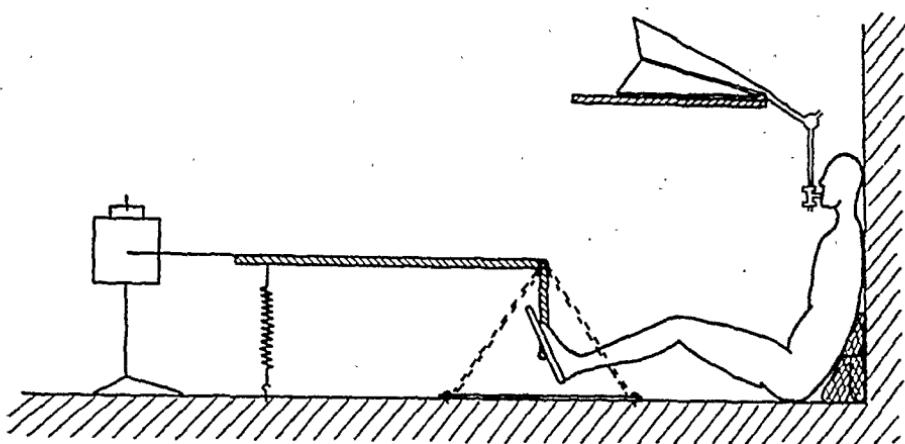


Fig. 2. Arrangement for the work experiments.

### Results and Discussion.

The results on subject E. A. (35 years, 172 cm, 70 kg) are presented in fig. 3. The ventilation and the alveolar  $pCO_2$  in rest and during the electrically induced and the normal voluntary work on the same machine, are plotted against the corresponding oxygen intakes. It can be seen from fig. 3, that the ventilation during the electrically induced work is increasing corresponding to the oxygen intake in the same manner as during the voluntary work. It can further be seen, that the alveolar  $pCO_2$  is the same during the two kinds of work, i.e. equal to the resting value of 39 mm Hg.

As mentioned in the introduction it has been shown (ASMUSSEN, CHRISTENSEN and NIELSEN 1943) that the increase in the excitability of the respiratory centre during muscular work is not due to substances produced in the working muscles, but must be supposed to be either of cortical or of reflex origin. In the experiments with electrically induced work here presented the mechanism of regulation seems to be intact in spite of the fact, that the cortical impulses are excluded and replaced by the "Myotensor". It must therefore be concluded that the increase in excitability of the respiratory centre is not brought about by irradiation of the cortical motoric impulses but must be of reflex origin.

This view of the regulation of respiration during muscular work is supported by experiments of HARRISON, CALHOUN and

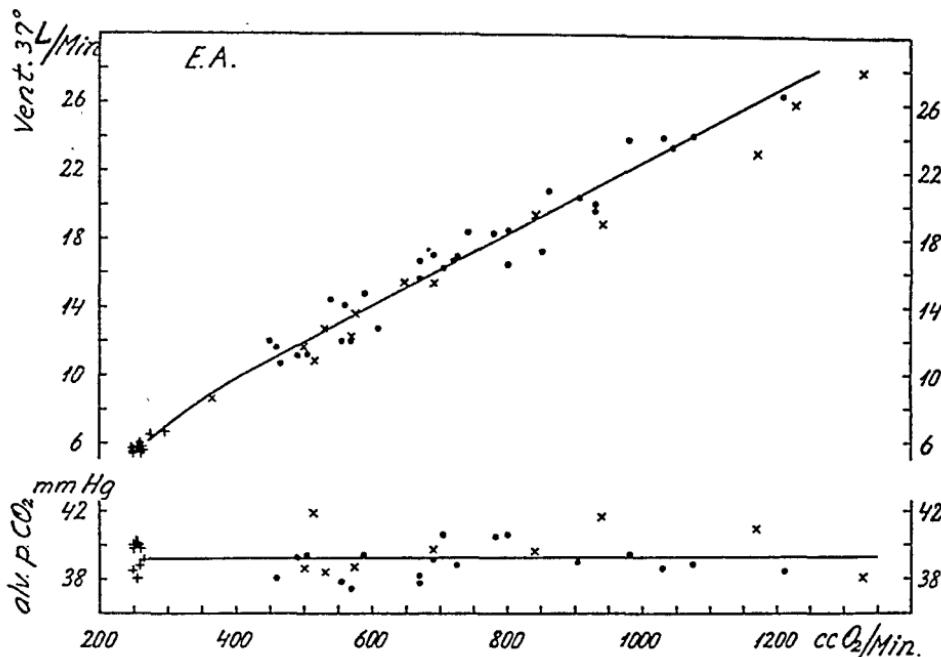


Fig. 3. Subj. E. A. Abscissae: O<sub>2</sub>-intake cc/min. Ordinates, upper curve: ventilation (37°, satur., prev.bar.press.) 1/min, lower curve alveolar pCO<sub>2</sub> mm Hg.  
 + resting values. ● electrically induced work. × voluntary work.

HARRISON (1932). These authors showed on dogs, that passive movements of hindlegs, which were only in nervous connection (through the sciatic nerve) with the rest of the body, caused a small increase in ventilation. They concluded from these experiments that "the increase in ventilation during 'mild' exercise is due — in part at least — to respiratory reflexes arising in the moving parts".

It was now likely to suppose, that the afferent impulses controlling the breathing during exercise were evoked in the kinesthetic endorgans controlling muscular movements, and led to the respiratory centre along the path of the kinesthetic sensations, e. g. in the posterior fascicles.

In order to study this question we made an extensive series of experiments on a subject suffering from tabes dorsalis. In this subject H. Th. (57 years old) all the kinesthetic sensations controlling muscular movements and limb posture were completely extinguished in the legs and in the lower part of the trunk and had been so for 7 years. Before his ailment H. Th. had been a hodman and had been of great muscular strength. Now he was of course completely untrained as far as his legmuscles were concerned.

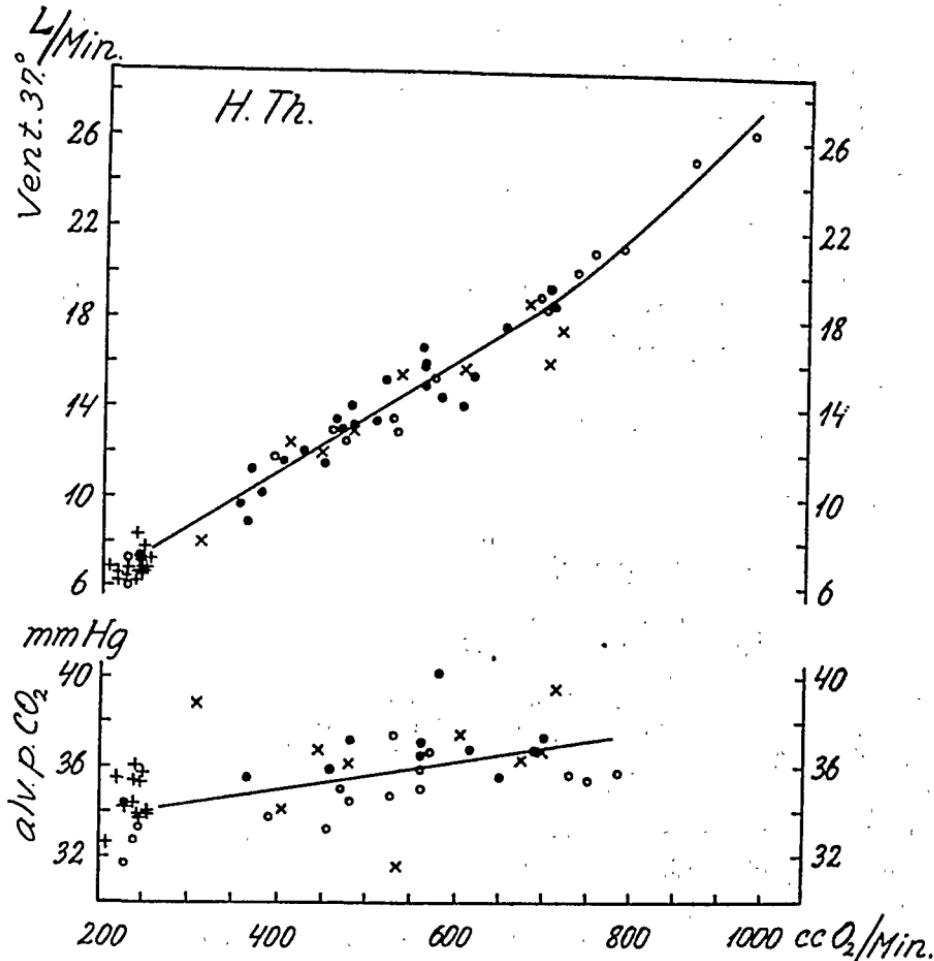


Fig. 4. Subj. H. Th. (Tabes dorsalis). Co-ordinates as in fig. 3. + resting values. ● electrically induced work. × voluntary work. ○ voluntary work on modified bicycle ergometer and corresponding resting values.

He came to the laboratory in his wheelcar. He was intelligent and made excellent service as a subject.

After a series of preliminary experiments in which the subject was practiced in the experimental technique, the experiments presented in fig. 4 were made. The fig. shows the ventilation and the alveolar  $pCO_2$  in relation to the oxygen intake from 3 kinds of work, viz. voluntary work and electrically induced work on the machine mentioned above (fig. 2) and voluntary work on a modified Krogh bicycle ergometer (NIELSEN 1938). To the astonishment of both the subject and ourselves it turned out that the subject worked very well on this bicycle, when his feet were tied to the pedals, and when he could watch the movements of his legs.

Probably the great inertia of the system was also essential for this ability. During the electrically induced work, in which the subject held his eyes closed, it was necessary that his knees were tied together and that an assistant lightly supported his legs to prevent them from falling to the side.

As is seen in fig. 4 the ventilation increases in the normal way corresponding to the oxygen intake both during the electrically induced work and during the two kinds of voluntary work.

In contradistinction to what was the case with the normal subject (see fig. 3) the alveolar  $pCO_2$  here is slightly increasing with increasing oxygen intakes. In order to evaluate the significance of this increase for the regulation of the ventilation, it was necessary to make a determination of the excitability of the respiratory centre of the subject, both at rest and during muscular work. In these experiments, which were carried out in the way described by NIELSEN (1936), the subject breathes air mixtures containing varying concentrations of  $CO_2$ . When a steady state of respiration is reached, simultaneous determinations of the pulmonary ventilation and of the alveolar  $pCO_2$  are made. The pulmonary ventilations from these experiments together with the values from the experiments in which the subject had been breathing normal atmospheric air, are plotted against the alveolar  $pCO_2$  in fig. 5. This fig. reveals the same characteristics as the curves published earlier by NIELSEN (1936), viz. a practically linear relationship between alv.  $pCO_2$  and ventilation, and a parallel shift to the left of the curve representing the results from the work experiments in relation to the resting curve. Compared with corresponding "excitability curves" from other subjects the slope of the curves in fig. 5 is rather small although it still appears to lie within the normal range.

Fig. 5 shows that to obtain an increase in the ventilation from about 7 l/min to about 21 l/min, both at rest and during the work, an increase in the alveolar  $pCO_2$  of about 17 mm Hg. is required, whereas a corresponding increase in the ventilation in the work experiments in fig. 4 was accompanied by an increase in the alveolar  $pCO_2$  of only 3 mm Hg. This slight increase, consequently, cannot have been of essential importance to the increase in ventilation observed during the voluntary and electrically induced work on H. Th. (fig. 4).

The results on H. Th., therefore, in the first instance confirm the conclusion reached above, viz. that the increase in excitability

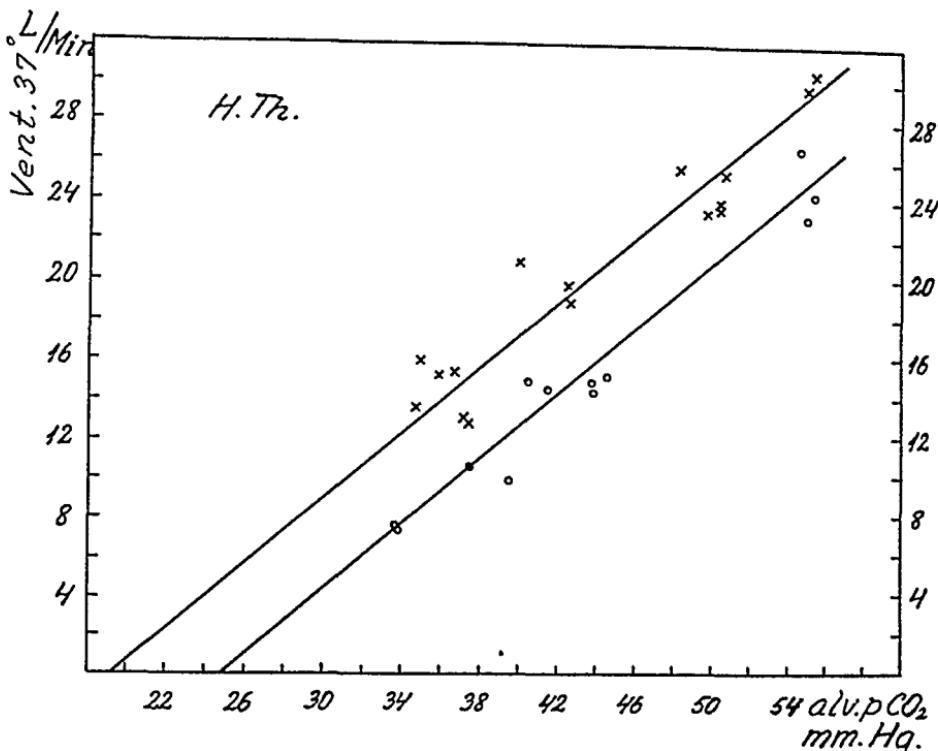


Fig. 5. Subj. H. Th. Excitability curves,  $\circ$  in rest and  $\times$  during work (120 mkg/min) on modified bicycle ergometer. Abscissae: alveolar  $pCO_2$ , mm Hg. Ordinates: ventilation ( $37^\circ$ , satur., prev.bar.press.), 1/min.

of the respiratory centre is not brought about by irradiation of cortical impulses, but must be of reflex origin. And the experiments on the patient with tabes dorsalis are perhaps still more convincing than the experiments on the normal subject, as the patient because of his ailment had no subjective apprehension of the intensity of the electrically induced work.

But secondly these experiments have shown that in spite of the fact, that this subject had no sensations or control of his muscular movements the regulation of the respiration during work acted in the normal way. From this it follows, that the reflexes controlling the ventilation during work, cannot be conveyed by the nervous tracts, which are known to be destroyed in tabes dorsalis, i. e. the posterior fascicles, but must be carried to the centre by sensory nerves outside this part of the medulla.

### Summary.

In experiments with voluntary and electrically induced work on a normal subject and on a patient (tabes dorsalis) in whom all the ordinary kinesthetic sensations were completely extinguished, the pulmonary ventilation increased during both kinds of work in the normal way i.e. corresponding to the oxygen consumption, in spite of the fact, that the cortical innervation during the electrically induced work was substituted by the apparatus for electrical stimulation.

From these experiments it is concluded, that the nervous impulses which increase the excitability of the respiratory centre, thus causing the rise in the ventilation during work, are not of cortical origin, but most probably must be brought about reflexly from the working muscles.

The experiments with the patient suffering from tabes dorsalis show further, that the reflex impulses must be carried to the centre through nerve paths outside the posterior fascicles, which are known to be destroyed in this disease.

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## CO<sub>2</sub>-breathing and Output of the Heart.

By

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Received 1 July 1943.

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During an earlier investigation (ASMUSSEN and KNUDSEN 1941) it was noticed incidentally that hyperventilation, induced by means of high concentration of CO<sub>2</sub> in the inspired air, produced an increase in the output of the heart when the subject was lying horizontally, but that the hyperventilation had no such effect if the subject was tilted passively to a position with the head upwards (+ 60°). It is the purpose of this paper to put this observation to a closer test and to make an attempt to find out the reason for the increased rate of circulation during hyperventilation in the horizontal position.

Earlier investigations along this line have brought contradictory results. LINDHARD (1915) and LILJESTRAND (1919) found no effect on the circulation during CO<sub>2</sub>-breathing. GROLLMAN (1930), however, stated that although concentrations up to 6 per cent CO<sub>2</sub> in the inspired air had no effect, still larger doses slightly increased the cardiac output of the heart. Voluntarily forced breathing had the same effect as also NORLIN (1932) found, and it was concluded, that the increase in cardiac output was due to mechanical effects of hyperventilation and not to a chemical effect of CO<sub>2</sub> itself.

The effects of forced breathing on the rate of circulation were well known. Already KROGH and LINDHARD (1912) noticed that the cardiac output during the period of forced breathing, which precedes a determination of the circulation rate after their meth-

od, was augmented. It was, however, generally assumed that this effect was of short duration, and first NORLIN (1932) demonstrated, that the effect could be maintained for more than one hour. As the cause of this effect an increased negativity of the intra-thoracal pressure seemed the most probable, but also the possibility of a pressing-out of blood from the abdomen was discussed. (KROGH and LINDHARD, GROLLMAN). NORLIN gives no explanation of the fact, but seems to believe, that at least part of the augmentation is due to some chemical influence caused by the shift to the left of the Hb-dissociation curve during the alkalosis of voluntary ventilation.

The effect of altered intra-thoracal pressures on the venous return to the right heart have been extensively reviewed by GOLLWITZER-MEIER (1932). The author sums up her review by stating, that the venous return in most cases is increased during inspiration and decreased during expiration, but that the two effects balance each other, so that the sum total should be no effect on the average circulation rate. In recent time HERBST (1940) claims to have demonstrated an inverted proportionality between the intrapulmonary pressure and the minute volume of the heart (Broemser-Rancke method), but ANTHONY, LENT and MÜLLER (1941) using the same method, find no effect of increased respiratory resistance on the circulation rate in ten subjects.

*Methods:* Varying degrees of hyperventilation were produced by different concentrations of CO<sub>2</sub> in the inspired air. Mixtures containing from 2.5 to 7.5 pct of CO<sub>2</sub> in atmospheric air were procured in cylinders and inspired through valve and mouthpiece. The expired air was collected in Douglas bags, measured and analyzed in the usual way. In some experiments the intrapulmonary pressure was varied by means of a resistance on the inspiratory or on the expiratory side of the valve. The resistance consisted of a large flask partly filled with water into which the inspiratory, respectively the expiratory, tube could be set to varying depths, thus giving a resistance of known magnitude.

After a preliminary period of at least 15 minutes the cardiac output was determined by means of the acetylene method according to GROLLMAN. The pulse rate, the arterial blood pressure and the respiratory frequency also were determined.

Most of the experiments were carried out on E. A. (35 years, 172 cm, 70 kg) others on A. H. (23 years, 175 cm, 69.5 kg).<sup>1</sup> All experiments were made in the morning after a resting period in the horizontal position of at least 1 hour.

<sup>1</sup> The experiments on A. H. were carried out by Mr. BOJSSEN MORTENSEN as part of his work for obtaining the degree of cand. mag.

*Results:* The result from a series of experiments on E. A. and A. H. breathing an air mixture containing about 6.5 pct CO<sub>2</sub> in the horizontal and in a tilted position (+ 60°) are averaged in table I, and compared with results from experiments in the normal condition.

Table I.

Subj.		Lying horizontally		Tilted 60°
		normal air	6.5 % CO <sub>2</sub> in insp. air	6.5 % CO <sub>2</sub> in insp. air
E. A.	O <sub>2</sub> -intake cc/min. . . .	238	261	239
	a.-v. O <sub>2</sub> -diff. cc/l . . . .	47 [6]	31 [2]	57 [2]
	Card. output l/min. . . .	5.07	8.42	4.18
A. H.	O <sub>2</sub> -intake cc/min. . . .	246	292	287
	a.-v. O <sub>2</sub> -diff. cc/l . . . .	47 [9]	35 [6]	69 [3]
	Card. output l/min. . . .	5.22	8.84	4.18

(Figures in brackets indicate number of determinations.)

The ventilation was in all the CO<sub>2</sub> experiments a little more than 30 l/min. It is obvious that the cardiac output increases during hyperventilation in the horizontal position. This increase is partly due to the increased O<sub>2</sub> intake, but as the arterio-venous O<sub>2</sub>-difference decreases, the increase in the output of the heart must have been greater than corresponding to the slight rise in the metabolic rate. Quite different are the results when the subjects were tilted. In this case the cardiac output is diminished, although at least in A. H. the O<sub>2</sub>-intake is still greater than in the normal condition. The arterio-venous O<sub>2</sub>-difference consequently is higher than normal. The decrease in cardiac output is of the same magnitude as that occurring when the subject, without breathing CO<sub>2</sub>, is tilted to the same position, so that in this case, the CO<sub>2</sub> and the hyperventilation have had no measurable effect on the cardiac output.

The effect of varying degrees of hyperventilation on the cardiac output and on the arterio-venous O<sub>2</sub>-difference of E. A. in the recumbent position is shown in fig. 1.

The average of the resting values (ventilations between 5 and 6 l/min) are for the cardiac output 5.3 l/min for the a.-v.

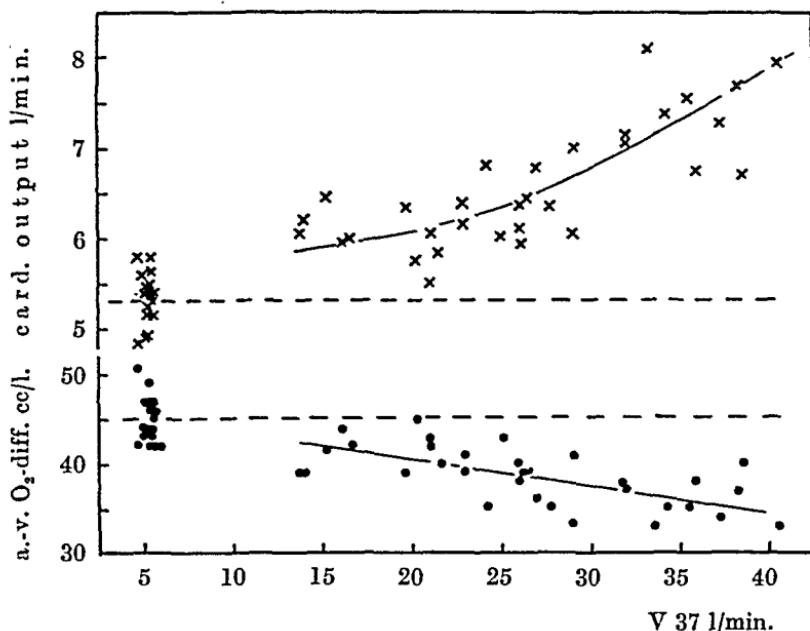
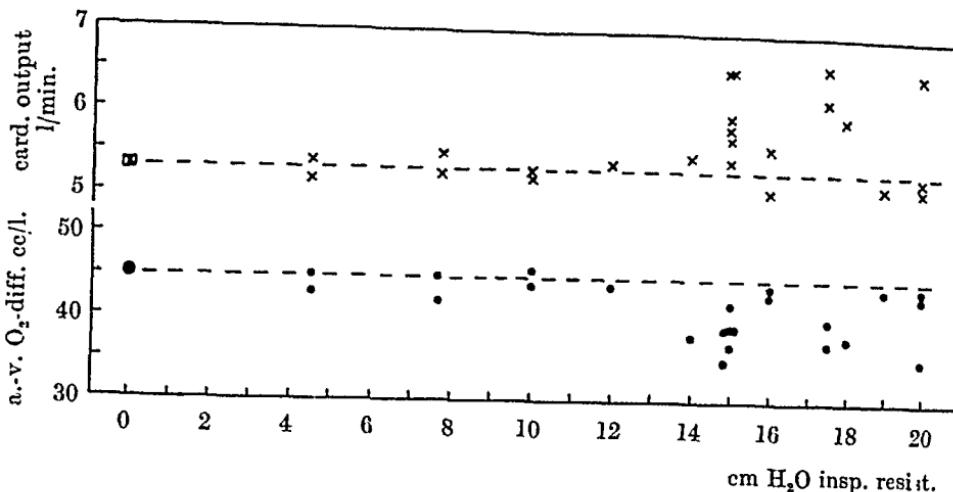


Fig. 1. Cardiac output and arterio-venous O<sub>2</sub>-difference during hyperventilation (CO<sub>2</sub>-breathing).

O<sub>2</sub>-difference 45 cc/l. The fig. shows, that with increasing ventilation there occurs an almost recti-linear decrease of the a.-v. O<sub>2</sub>-difference. The cardiac output increases with increasing ventilation, but more than corresponding to the decrease in a.-v. O<sub>2</sub>-difference. This is due to the fact, that the O<sub>2</sub> intake increases with increasing hyperventilation. The pulse rate was only slightly higher than normal in the experiments with CO<sub>2</sub>-inhalation so that the stroke volume of the heart on an average increased with the ventilation. This increase of stroke volume, however, was far less regular than the increase in the cardiac output. Also the arterial blood pressure rose slightly, but only with the highest degrees of hyperventilation (7 pct CO<sub>2</sub> in insp. air) the rise in systolic pressure reached a magnitude of 10 mm Hg.

The results from the experiments in which the inspiratory resistance was increased are presented in fig. 2. It shows, that up to an extra inspiratory resistance of 12 cm of water the a.-v. O<sub>2</sub>-difference and the cardiac output are unaffected, and that still higher resistances (up to 20 cm H<sub>2</sub>O) have only slight and rather irregular effect on the cardiac output and the a.-v. O<sub>2</sub>-difference. The ventilation/minute and the O<sub>2</sub>-intake in these experiments were within the normal range of the resting experiments, and



*Discussion:* The results from the experiments with hyperventilation in different postures show, that the CO<sub>2</sub> has not acted as a chemical stimulus for the circulation. If that were the case it is not conceivable why it should have had no effect in the tilted position, as other chemical stimuli for the circulation are known to act also in such case (e. g. low arterial pO<sub>2</sub> (ASMUSSEN and KNUDSEN 1941)). It must therefore be concluded, that the effect of the CO<sub>2</sub> breathing on the cardiac output is a mechanical effect of the hyperventilation as also GROLLMANN (1930) and others assumed. The fact, that CO<sub>2</sub>-inhalation does not increase the cardiac output when the subject is in an erect position, possibly explains the discrepancies of earlier investigations, as the position of the subject apparently has not been identical in the different investigations.

In contradistinction to GROLLMAN's results the present experiments show that even low concentrations of CO<sub>2</sub>, causing relatively small degrees of hyperventilation, have an unmistakable effect on the circulation. It seems possible then, to increase the rate of circulation of a subject gradually up to more than 50 pct by letting the subject inspire CO<sub>2</sub>-mixtures, thus hitching the mechanical force of respiration to the circulatory mechanism. The lowered a.-v. O<sub>2</sub>-difference and the increased pCO<sub>2</sub> of the blood will raise the mean oxygen tension of the venous blood, and hence of the tissues. It is well possible, that the beneficial effect of carbogen (HENDERSON 1938) in cases of peripheral circulatory failures may be due, partly at least, to this effect.

As an explanation of how an increased ventilation does augment the cardiac output the increased negativity of the intra-thoracal pressure during the deep inspirations has been mentioned. Such a negative pressure is assumed to aid the venous return of blood by creating a greater fall of pressure from the veins outside the thorax to the right heart.

The intra-pleural pressure is in most text-books of physiology since the days of DONDERS given as — 3 to — 5 mm Hg. in the normal expiratory phase and — 5 to — 10 mm Hg. at the end of a normal inspiration. After a maximal expiration it should be — 2 mm Hg. and after a maximal inspiration — 30 mm Hg. It is possible from these data to draw a curve approximately representing the negativity of the intra-pleural pressures at different fillings of the lungs. The curve will be of a hyperbolic shape, rising rapidly with increasing filling of the lungs. From such a curve,

assuming that the mean capacity of the lungs is practically unchanged during hyperventilation (as found by ASMUSSEN and CHRISTENSEN (1939) during work) it is possible to estimate the mean negativity of the intra-pleural pressure at the different respiratory depths found in the experiments. A curve representing these values plotted against the cardiac outputs and the a.-v. O<sub>2</sub>-differences found in the experiments of fig. 1, shows that these two functions increase, respectively decrease, in a very regular manner with increasing negativity of the intra-pleural pressure. But, if one tries to make the experiment of fig. 2 and fig. 3 fit into the picture, one fails.

In the experiments represented by figs. 2 and 3 the pressure inside the lungs during inspiration, respectively expiration, must have been approximately so much lower, respectively higher, than the atmospheric pressure as the inspiratory or expiratory resistance indicate.

From this follows, that the mean intra-pleural pressure in the experiments with increased inspiratory resistance must have been lowered by approximately half the value of the inspiratory resistance, and in the experiments with increased expiratory resistance increased by about half the value of the expiratory resistance.

If the results in figs. 2 and 3 are treated according to this conception and plotted with the same co-ordinates as mentioned above for the results of fig. 1, the resulting curves show, that increases in the negativity of the intra-pleural pressures far exceeding the largest values found in the CO<sub>2</sub>-experiments, are still without any effect on the cardiac output, and that the very low values of the negativity of intra-pleural pressures found in the experiments with increased expiratory resistances (fig. 3) instead of giving a lowered cardiac output, as should be expected, show a tendency to make it increase.

It seems justifiable then to conclude from these experiments, that the increased negativity of the intra-pleural pressure cannot be the reason for the increased rate of circulation in the experiments with hyperventilation.

If the blood is not "sucked" back to the heart with increased ventilation, the possibility remains that the large respiratory movements in some way or other increase the venous return to the heart.

The large excursions of the diaphragm during hyperventilation will increase the intra-abdominal pressure. This in turn will create

a greater pressure head for the venous blood coming from the abdomen and thus "squeeze" the blood out of the abdominal veins (especially in the liver) into the thorax (see GOLLWITZER-MEIER (1932)). The deeper the inspirations, the more effect they should have. The experiments with CO<sub>2</sub>-breathing (fig. 1) also show a recti-linear correlation between a.-v. O<sub>2</sub>-difference and respiratory depth, when these two functions are plotted against each other. On the other hand, if the blood is pressed out of the abdominal veins (liver veins) it needs a special explanation to understand, why this is not the case also in the tilted position (table 1).

There exists, however, still another possibility to explain how the deep respirations during hyperventilation in the horizontal position might increase the output of the heart: The deep respirations may increase the venous return to the left heart by pressing out blood from the vessels of the lungs themselves. Certain observations made by ASMUSSEN, CHRISTENSEN and SJÖSTRAND (1939) may have a bearing on this possibility. These authors found, that 3—4 deep respirations in the lying-down position would increase the vital capacity of the subject by 210 cc, and they explained this as a result of a pressing-out of such an amount of blood from the thorax during the deep respirations. They could further show, that if the lungs beforehand were "emptied", deep respirations in the horizontal position caused only a very slight increase in the vital capacity (70 cc). (The subject was first placed in a tilted erect position. Inflated cuffs around the thighs hindered the blood, accumulated in the lower extremities, in returning to the central vessels, as the subject reassumed the horizontal position.) These experiments show, that it is possible to drive out blood from the thorax by deep respirations, if the lungs contain a surplus of blood (as they do in the horizontal position) not, however, if the lungs are deprived of this surplus (as in the tilted, head-upwards position). This observation explains why hyperventilation has a marked effect on the output of the heart in the horizontal position, and none in the upright, tilted position, if one assumes, that the amount of blood that can be driven out of the thorax by the large respirations is kept circulating and is not stored away somewhere else in the body.

Also for the assumption, that deep respirations may drive out blood from the abdominal vessels, thus helping the return flow of blood to the heart, the different distribution of blood between lower extremities and the rest of the body, which exists in the two

positions here discussed, may play an important rôle, viz. if the driving-out of blood from the abdomen is depending on a surplus of blood in the liver veins. It is well possible, that such a surplus exists in the lying position only and that in the tilted position it is transferred to the veins of the lower part of the body.

Whether the blood, which accounts for the increased cardiac output during hyperventilation, comes from the lungs themselves or is pressed out of the abdominal veins, a surplus of blood in the upper part of the body seems to be of importance.

In order to test this assumption two series of experiments were made in which the subject breathed CO<sub>2</sub>-mixtures in the lying-down position, in one series after having been placed in the upright tilted position for a few minutes, in an other after having been tilted to a head-down position for a few minutes. In both cases cuffs around the thighs of the subject were inflated just before the subject was returned to the horizontal position, in order to maintain the abnormal distribution of blood between the lower extremities and the rest of the body, which exists in the two tilted positions.

After 10 to 15 minutes of hyperventilation by CO<sub>2</sub> the cardiac outputs were determined.

The results, given in table II, indicate, that hyperventilation tends to give a higher cardiac output and a lower a.-v. O<sub>2</sub>-difference when the legs are "empty" than when they are "full" of blood.

Table II.

	Legs "empty"			Legs "full"		
	V <sub>37</sub> l/min.	a.-v. O <sub>2</sub> diff. cc/l	Card. output l/min.	V <sub>37</sub> l/min.	a.-v. O <sub>2</sub> diff. cc/l	Card. output l/min.
	15.3	35	6.75	15.6	45	5.37
	24.8	36	6.35	26.5	43	5.47
	26.5	34	7.00	28.8	41	5.34
	27.0	37	6.59	31.9	41	5.73
	34.3	36	7.50	35.5	36	6.94
	34.7	31	8.03	38.0	37	6.52
	37.4	40	6.00	38.1	39	6.95
	38.3	27	10.00			
Average	29.8	34.5	7.28	30.6	40.8	6.05

The main results and conclusions of the present investigations then are: Hyperventilation in the lying-down position, caused by CO<sub>2</sub>-breathing, gives an increased cardiac output with a concomitant lowered a.-v. O<sub>2</sub>-difference. As this only occurs in the horizontal position, not in the tilted, head-upwards position it is concluded, that the effect is not brought about by a chemical action of the CO<sub>2</sub>, but that it must be caused by the mechanical forces of hyperventilation. As even large changes in the intra-pleural pressure, created by means of increased inspiratory or exspiratory resistances, has but slight and irregular effects on the circulation rate, it is concluded, that the increased cardiac output during prolonged hyperventilation is not due to an increased "sucking back" of the blood to the thoracic veins.

It is put forward as a hypothesis, that the distribution of blood plays an important rôle for the increase in the cardiac output during hyperventilation: If the lungs are rich in blood (as in the horizontal position) deep respirations will drive out blood from them (indicated by the rise in vital capacity) thus creating an increased flow of blood to the left heart. If the lungs are deprived of their surplus of blood (tilted position) no such increase can be produced. The same may be said concerning a pressing-out of blood from the abdomen during deep inspirations, if one assumes, that also the blood-filling of the upper part of the abdomen is influenced by posture. Whereas the different blood-fillings of the lungs in different postures can be measured by means of the vital capacity, no such measurement is available for different blood-fillings of the abdominal vessels.

### Summary.

On two subjects it is shown, that hyperventilation, caused by CO<sub>2</sub>-breathing, has an increasing effect on the cardiac output if the subject lies horizontally, none, however, if the subject is tilted to an upright (60°) position. It is concluded, that the effect of CO<sub>2</sub>-breathing on the cardiac output must be due to the mechanical forces of hyperventilation and not to a chemical action of the CO<sub>2</sub> on the circulation, as otherwise the difference of the effect in the two positions is unexplained.

By increasing the inspiratory or exspiratory resistance artificially the negativity of the intra-pleural pressure could be increased, respectively decreased. It was found that this had very

slight and irregular effects on the cardiac output, and it is concluded, that the augmented negativity of the intra-pleural pressure during hyperventilation cannot be the cause of the increased cardiac output.

It is discussed how the deep respirations of the hyperventilation might influence the cardiac output, and the conclusion is put forward tentatively, that this is possible if one assumes that the enlarged respiratory movements cause a pressing-out of blood from the intra-pulmonary (or, and from the abdominal) vessels, when these are "filled" with blood (as in the horizontal position), not, however, when they are "empty" (as in the tilted position). The conclusion is supported by experiments, in which part of the blood was shut off in the legs.

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## Disturbance of Circulation in Convulsions of the Epileptic Type.

### IV. Animal Experiments.

By

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Received 26 May 1943.

In 1887 d'ARSONVAL advanced the opinion that industrial electric currents affecting bulbic centres might produce a reversible inhibition of respiration and of the heart's action. The great importance of this in connection with electric shocks has also been recognized in later times. — PREVOST and BATELLI (1899) were able to show by animal experiments that a short electric shock (mouth — rectum) induced convulsions, with an initial decrease in blood pressure accompanied by a cardio-inhibition, and that after a few seconds the heart started to beat again and the blood pressure to increase. BATELLI (1903) found that a short electric shock (45 periodic A. C. of 120—140 volts for 0.05 sec.) sent through the head of a dog induced a typical epileptic convulsion. During this convulsion there is a marked initial fall of blood pressure and cardio-inhibition, which disappeared after vagotomy, as shown by BINI and PUDDU (1941), who also established that the blood pressure in vagotomized animals increased considerably, even during the initial phase of the convulsions.

LOUGHEED and HALL (1939), studying cardiazol convulsions in the rabbit, found that a marked rise of blood pressure preceded the tonic phase of the convulsion. This rise, which may be partly due to a cardiazol effect, was not less marked in curarized animals. From this they concluded that the convulsions *per se* had little significance for the disturbances of the circulation occurring during the attack. This seems also to conform with the view held by BINI and PUDDU.

According to previous investigations in this series, the intra-thoracic and intra-abdominal pressures rise considerably during clinical electroshock convulsions, causing marked disturbances of circulation similar to those believed to occur in Valsalva's experiment, with a rise of blood pressure and a decrease in cardiac output.

In the following paper we attempt to analyze the effect of muscle convulsions on the circulation.

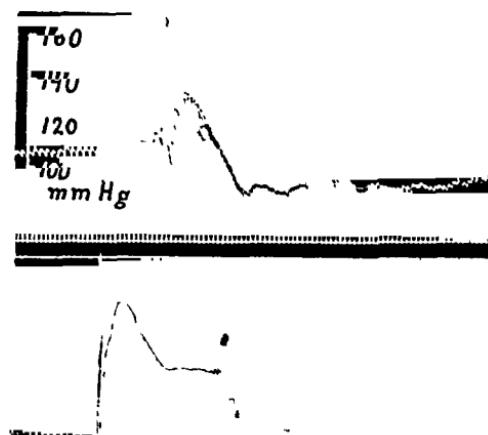


Fig. 1. Rabbit, 2.3 kg.

From above: arterial blood pressure, time in secs., signal marking the shock (140 V., 0.3 sec.), intra-abdominal pressure (maximum height 20 mm Hg).

*Methods.* Rabbits were used as experimental animals. Cannulae were inserted in the carotid artery, the pleural cavity and the abdomen, under local anaesthesia.

The cannula inserted in the pleural cavity was a rather wide tube with a transverse plate 5 mm from the point. A short incision was made in the skin, and a small area between two ribs was so prepared that the fascia and the muscles could be sewn around the plate, hermetic closure being secured. The skin was subsequently sewn round the plate, and the cannula thus became firmly fixed to the chest wall, any change in its position during the convulsion being prevented. The cannula was short, wide, and blunt-ended, to prevent injury to the lung. The intra-abdominal pressure was recorded from a small rubber balloon enclosed in the abdominal cavity and attached to a cannula through the abdominal wall. The pressures were registered by means of Marey tambours.

In our earlier experiments the convulsions were induced by intravenous injection of cardiazol, but later electroshock was employed.

The electrodes were covered with cotton wool, soaked in RINGER's solution, and inserted in the rabbit's ears.

For the study of the heart rate, a fine needle was inserted in the heart wall through the chest wall, and its movements were observed. An opening of the thorax could be avoided in this way.

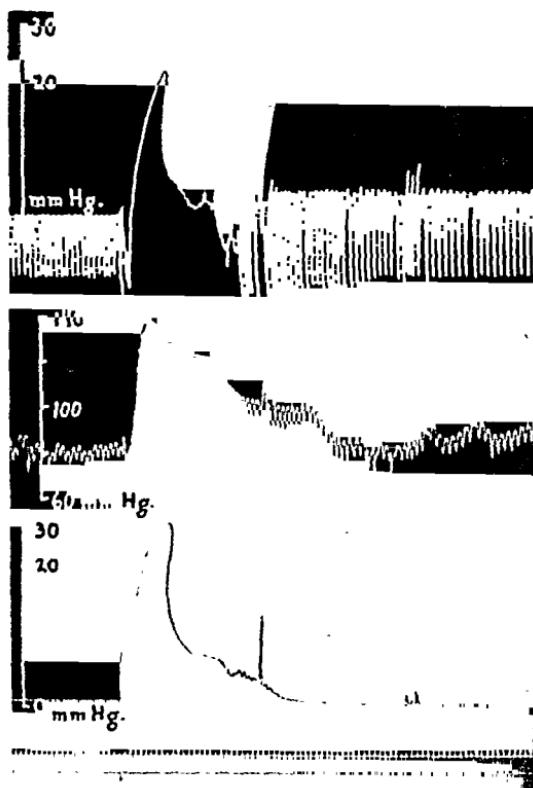


Fig. 2. Rabbit, 2.1 kg.

From above: intrathoracic pressure, arterial blood pressure, intra-abdominal pressure, time in secs., and signal, marking the shock (140 V., 0.8 sec.).

*Results.* If the electric current be sufficiently strong — we used 50 periodic A. C. of 140 volts with a duration of 0.2—0.3 sec. — the rabbit passes into a tonic-clonic convulsion of the epileptic type. The whole convulsion usually lasts 35—40 sec., the tonic phase passing in about 10 sec. After the convulsion the animal is not particularly exhausted but should be allowed to recover for about an hour, if a fresh shock is to be induced with good results.

During the first part of the tonic phase of the convulsion, the blood pressure does not rise, sometimes there is even a fall. The pulse oscillations disappear. In the later part of the tonic

or at the beginning of the clonic phase, the blood pressure rises by jerks, reaching a moderate height. At the end of the convolution, the blood pressure gradually falls below the initial pressure (Fig. 1). Only exceptionally is there an initial rise (Fig. 2).

The needle in the heart always showed satisfactory oscillations before the shock. At the onset of the attack the needle

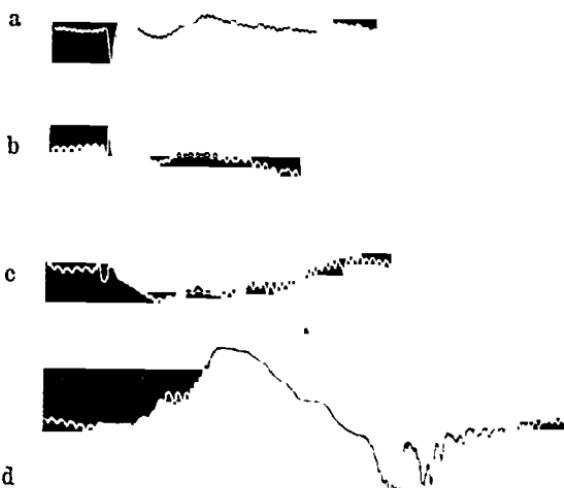


Fig. 3. Rabbit, 2.2 kg.

a) 55 V., 0.1 sec. (no convulsions). b) 80 V., 0.1 sec. (no convulsions). c) 140 V., 0.1 sec. (no convulsions). d) 140 V., 0.2 sec. (convulsions).

ceased to oscillate regularly and showed no, or only a few oscillations during the 10 seconds during which the tonic phase lasts. This bradycardia subsequently changed into a moderate tachycardia at the end of the attack. The total inhibition of the heart's activity closely corresponded to the loss of pulse oscillations and the absence of rise in the blood pressure record during the earlier part of the convolution.

The intra-thoracic pressure rose from a few to 25 mm Hg during the shock. The pressure usually increased rapidly during the first part of the tonic phase and then successively decreased with the onset of the clonic phase (Fig. 2). Sometimes an initial sharp rise and a rapid return was observed.

The intra-abdominal pressure showed changes similar to the intra-thoracic pressure, but the rise was more constant and somewhat greater. On an average the pressure rise amounted to 10—30 mm Hg, and the rise was more pronounced during the tonic phase (Fig. 2).

A sub-threshold electric shock usually led to a temporary fall of blood pressure with a simultaneous inhibition of the heart's action (Fig. 3).

In atropinized animals (5 mg pr kg body weight intramuscularly) the cardiac inhibition was eliminated, and during the convulsion there was a moderate tachycardia. The arterial blood

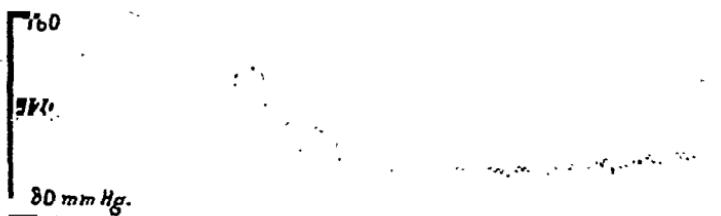


Fig. 4. Rabbit, 2 kg.

3 min. before the shock 10 mg. atropine was injected intravenously. From above: arterial blood pressure, time in secs., and signal marking the shock (140 V., 0.2 sec.).

pressure rose immediately on the onset of the convulsion (Fig. 4). The secondary fall of blood pressure sometimes became less pronounced.

The same results were obtained with curarized animals. There was no cardiac inhibition, the heart showing only an initial and temporary, moderate bradycardia, which may have been caused by the electric current. The blood pressure rose immediately, and in this case too there was a reduced secondary fall of blood pressure. Fig. 5 shows an experiment on a curarized animal, where the intra-abdominal pressure is registered in order to demonstrate the complete curarisation.

*Discussion.* During the convulsion induced by electro-shock there is usually an initial cardiac inhibition. After about 10 seconds, corresponding to the tonic phase of the convulsion, the heart starts to beat again and at the same time the arterial blood pressure increases. This increase is fairly moderate, amounting

on an average to 40—60 mm Hg. In atropinized animals the inhibition of the heart is done away with, and the rise of blood pressure starts at once, which permits of the conclusion that the initial heart slowing is due to vagus stimulation. This conforms with the results obtained by previous authors, though they considered the initial inhibition to have been due to a direct vagus stimulation owing to the electric shock.

We have, however, found that curarisation of the animals also prevents cardiac inhibition, the animal showing only a mo-

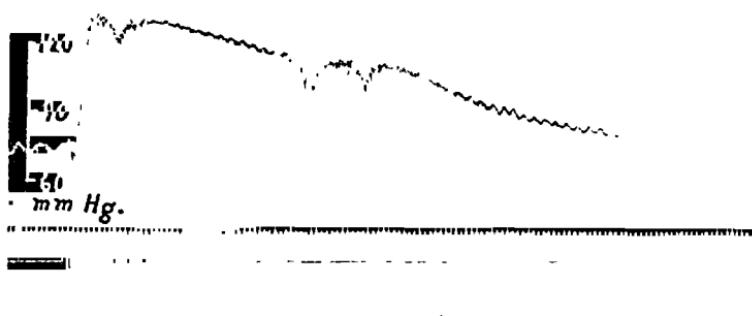


Fig. 5. Rabbit, 2.3 kg.

10 min. before the shock 7 ml of an 1 % curare solution was injected intravenously. Artificial respiration.

From above: Arterial blood pressure, time in secs., signal marking the shock (140 V. 0.3 secs.), intra-abdominal pressure.

derate bradycardia. Unless it is assumed that curare in this case inhibits the vagus centre, it seems most plausible to suppose that the convulsion produces some effect responsible for the cardiac inhibition. The moderate rise of intra-thoracic and intra-abdominal pressures during the convulsion must cause an increase in the pressure of the cerebrospinal fluid, and it seems probable that this increased intra-cranial pressure is the cause of the vagus stimulation.

Possibly the electric shock in itself gives a moderate vagus stimulation, with bradycardia. The cardiac inhibition in convulsions is thus probably the combined result of the direct electric stimulation and the muscle convulsions.

The rise of blood pressure in convulsions could be supposed to result on the increased intra-abdominal and intra-thoracic pressures, since this is known to produce such an effect. As the

rise of blood pressure, however, occurs even in curarized animals, straining cannot be the cause. As to a direct effect of the electric current, this usually leads to a fall of blood pressure, if the strength of the current be below the convulsion threshold. Thus it seems most probable that the rise in blood pressure is caused by some kind of cerebral excitation elicited by the seizure-promoting shock. The stop of the heart as observed in rabbits during the tonic phase of the convulsions would however counteract the tendency to a rise in blood pressure.

This change in circulation differs essentially from the conditions in man. During the clinical electro-shock the intra-abdominal pressure immediately rises to a high level (up to 220 mm Hg) (GORDH and SILFVERSKIÖLD, 1943), and this must cause considerable disturbances of circulation. During the major part of the tonic phase there is, however, a marked tachycardia, and the blood pressure rises immediately on the onset of the attack. Only exceptionally is there a cardiac inhibition of some duration. Thus the straining effect during the convulsions seems to dominate the picture in man, the vagus stimulation being less pronounced.

According to PENFIELD (1933), a cessation of pulsation in the cerebral arteries occurs during the epileptic convulsions. In man this cessation may be due to a Valsalva effect on the circulation with a decrease in the stroke volume, combined with active arterioconstriction (SILFVERSKIÖLD and ÅMARK, 1943). In the rabbit such a cessation may also be caused by the cardiac inhibition during the tonic phase.

### Summary.

In electrically induced convulsions in the rabbit, initial cardiac inhibition and bradycardia occurs. This can be prevented by atropine and is attributed to vagus stimulation. The rise in arterial blood pressure starts in the later part of the tonic or on the onset of the clonic phase.

Curare also prevents the cardiac inhibition, and the blood pressure rises at the beginning of the tonic phase. It is believed that the vagus stimulation is caused by a rise in intra-cerebral pressure, induced by the rise of the intra-abdominal and intra-thoracic pressures during the convulsion, this action being prevented by curarisation.

As the rise of blood pressure occurs even in curarized animals, it is not caused by the muscle convulsions.

The disturbances of circulation in rabbits induced by electro-shock differ in various respects from those found during electro-shock convulsions in man.

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## The Renal Excretion of Fructose.

By

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Received 27 May 1943.

While the excretion of glucose with the urine has by now been pretty thoroughly investigated, little work, and that little satisfying, has been done on the subject of the excretion of the other monohexoses. The renal reabsorption of glucose has been studied by SHANNON and FISHER (1938) who have measured the glucose and creatinine clearance of the dog simultaneously, using arterial blood for the analyses and keeping the glucose concentration of the plasma constant by intravenous infusion. The purpose of this work has been to investigate the renal output of fructose, using a similar technique, and especially to make it clear if the reabsorption of fructose is influenced by the amount of glucose, which is to be reabsorbed.

### Methods.

Fructose was determined by a somewhat modified RADT's method (1928). To 1.00 ml of the solution the fructose content of which is to be determined, 1 ml of 25 per cent HCl and 0.1 ml 20 per cent alcoholic solution of diphenylamine are added in test tubes. The tubes are placed in a boiling water-bath for precisely 15 minutes and are then cooled by immersion in an ample quantity of cold water. Then 2.00 ml of isoamyl alcohol is added and the precipitate of diphenylamine together with the blue compound is dissolved in it by energetic shaking for a few minutes without heating. The aqueous layer is pipetted off, and the analyses are placed in an ice-box for 4 or 5 hours before the intensity of the colour is measured in the Pulfrich photometer, filter S 66 and 5 mm cuvettes. The analysis being carried out in this way, the aftercolouring is negligible. The calibration curve is very slightly curved.

Analysis of serum: The blood sample is centrifuged in an ice-box immediately without any addition, the proteins are then precipitated according to HERBERT (1938) and the analysis is carried out on 1.00 ml

of filtrate. In blood from fasting dogs a blind of 3–4 mg per cent was found; the error from this source was eliminated by placing an analysis on serum taken before the infusion of fructose in the cuvette of reference. By analyses of plasma a coefficient of variation of 3.6 per cent was found. In all cases double determinations were made, and when the results deviated more than 5 per cent, the analyses were repeated.

Analysis of urine: Here a high blind may be found; this is small however under inanition, so the dogs were fasted for 18 hours before each experiment. Under these conditions the blind corresponds to an excretion of about 0.10 mg fructose per minute; the error produced by not correcting for this will only be important in experiments with very low fructose concentrations.

Creatinine was determined according to AXEL THOMSEN's principles (1938), glucose according to HAGEDORN-NORMAN-JENSEN, only the usual zinc-precipitation was not employed. Instead of this the analyses were carried out on the FOLIN-WU filtrate (as in the case of the galactose analysis of RAYMOND and BLANCO (1928)). By this modification both a smaller blind and a greater accuracy were obtained. The usual table might be used.

### Experimental Procedure.

The experiments were carried out on dogs. A constant fructose concentration in the blood was secured by intravenous infusion of a fructose-creatinine solution, starting 30—60 min. before the collection of blood and urine samples. The urine was collected from one or two periods of about ten minutes. At the mid-periods samples of arterial blood were taken, and from the amounts of fructose and creatinine excreted per minute and the concentrations in the blood the clearances were determined.

The infusion took place in the internal jugular vein by means of a drop infusion apparatus, the velocity of infusion being determined by the resistance of inserted capillaries. To facilitate the taking of arterial blood, the internal carotid had been previously provided with a silver jacket, from which a small silver tube led to the skin. By inserting the cannula through the tube the blood samples were easily obtained. The urine was collected by means of an inlying catheter, the bladder being washed with three times 20 ml of water both before and after the periods. The last wash fluid was added to the urine prior to the final dilution for analysis. The time from the end of the first rinsing to the end of the second was measured with a stop-watch.

All of the here cited experiments were carried out on a normal female dog, weighing 8.6 kg.

### Results.

*Simultaneous fructose and glucose excretion.* The object of the first experiments was to throw light upon the question whether the fructose-creatinine clearance ratio depends upon the amount

of glucose simultaneously reabsorbed. Table 1 shows the results of one of these experiments.

Table 1.

*Relation of the Fructose Excretion to the Concentration of Glucose in the Filtrate.*

	Infusion: Fructose-Creatinine		Infusion: Fructose-Glucose-Creatinine
	1. Period	2. Period	
Creatinine clearance .....	27.8	26.4	31.8
Fructose serum mg per cent .....	58.0	56.2	52.8
" reabs. mg per min. ....	8.3	8.1	8.25
Fr-Cr. clearance ratio .....	0.485	0.465	0.460
Glucose serum mg per cent .....	138	138	343
" urine mg per min. ....	0.2	0.13	21.9
" reabs. mg per min. ....			94
" threshold mg per cent .....			295

In the first part of the experiment a solution containing 15 g of fructose + 1.5 g of creatinine in 200 ml of water was infused with the velocity of 3.0 ml per min. The preliminary infusion lasted for 40 min., then the urine was collected from two consecutive periods of 10.83 and 12.50 min. In the second part the solution infused contained 12 g of fructose + 0.8 g of creatinine + 20 g of glucose in 150 ml of water. The velocity of infusion was unchanged and after a preliminary infusion lasting 40 min. the urine was collected from one period of 12.08 min.

In the first part a fructose-creatinine solution was infused, in the second part a solution of the same fructose concentration but with an ample quantity of glucose too. It appears from Table 1 that the creatinine clearance increased and the fructose concentration decreased a little from the first to the second part of the experiment. As shown by later experiments on the clearance ratios at different fructose concentrations in the blood, a small change in the concentration has in itself no measurable influence on the clearance ratio.

In the first part of the experiment the glucose concentration of the serum is 138 mg per cent and no demonstrable quantity of glucose is excreted with the urine. In the second the glucose concentration is 343 mg per cent, and some glucose is excreted, the serum concentration being somewhat higher than the threshold value, which may be calculated according to SHANNON and FISHER as 295 mg per cent. None the less no measurable dif-

ference between the fructose-creatinine clearance ratios can be demonstrated.

*Experiments at different urine flows.* With the technique here employed it was difficult to work with quite small urine flows, as a certain amount of water had to be infused, and besides the experimental error will be great when the urine flow is very small. It was considered more appropriate to compare moderate urine flows with very large ones produced by infusion of sulphate. The results of one of the experiments are given in Table 2.

Table 2.  
*Relation of the Fructose Excretion to the Urine Flow.*

	Infusion: Fructose-Creatinine		Infusion: Fructose-Sulphate Creatinine	
	1. Period	2. Period	1. Period	2. Period
Urine flow ml per min. ....	0.5	0.5	9.9	12.8
Creatinine clearance .....	32.2	39.4	36.6	40.0
Fructose serum mg per cent ..	29.6	29.0	25.0	24.4
» reabs. mg per min. ...	5.98	7.2	5.76	5.36
Fr.-Cr. clearance ratio .....	0.374	0.368	0.370	0.153

1. Part: A solution containing 7 g of fructose + 2 g of creatinine in 420 ml of water was infused with a velocity of 6.4 ml per min. Preliminary infusion for 45 min., then two periods of 11.0 and 10.0 min.

2. Part: Infusion liquid: 5 g of fructose + 0.8 g of creatinine + 30 g of  $\text{Na}_2\text{SO}_4$  in 300 ml of water. Velocity: 6.4 ml per min. Preliminary infusion for 20 min., then two periods of 9.50 and 8.17 min.

In the first part of the experiment the urine flow is about 0.5 ml per min. (of course it could only be measured very inaccurately). This may be a considerable diuresis for a dog of the size in question, but in the second part, where a quantity of  $\text{Na}_2\text{SO}_4$  was added to the infusion liquid, we nevertheless obtained a diuresis of more than twenty times this amount. In the first period of this division the clearance ratio was unchanged, while in the second a slight increase was found, which may exceed the experimental error.

*Experiments at different concentrations in the serum.* In Table 3 are given the main results of a series of experiments on the fructose excretion at different serum concentrations.

The time of the preliminary infusion in all cases exceeded 30 min. It is evident, that the fraction of the filtered fructose found in the

Table 3.

*Relation of the Fructose Excretion to the Concentration of Fructose in the Serum.*

Creatinine clearance	Fructose in serum mg per cent	Clearance ratio	Fructose reabs. mg per min.
30.1	3.5	0.285	0.75
31.4	4.9	0.232	1.19
29.5	5.1	0.233	1.15
44.6	6.0	0.223	2.08
46.8	26.4	0.360	7.94
39.4	29.0	0.368	7.20
32.2	29.6	0.374	5.98
35.9	49.2	0.520	8.49
37.4	51.6	0.452	10.56
26.4	56.0	0.465	8.10
27.8	58.0	0.485	8.30
41.0	58.0	0.572	10.2
50.6	61.6	0.542	14.3
41.8	71.2	0.650	10.4
44.2	86.0	0.474	20.0
33.2	320	0.77	24.6
38.2	336	0.79	27.0

urine increases with increasing concentration in the blood, but even at extremely low concentrations a considerable part of the fructose is excreted. No certain maximum of reabsorption could be demonstrated at the concentrations examined, but at the highest values given in the table, the amount of fructose reabsorbed only increased very little. The values of creatinine clearance are included in the table in order to show its variations, which must have increased the deviation of the results to some degree. It must be remembered, however, that unlike the determination of the clearance ratio, the determination of the creatinine clearance is encumbered with errors arising from the fact, that the time of secretion of the urine collected cannot be measured accurately. Thus the creatinine clearance is likely to have varied somewhat less than shown in the table.

### Discussion.

Our investigations confirm the recorded findings in the literature on the subject to the effect that by intravenous infusion of a fructose solution fructose is found in the urine. Furthermore the experiments show that fructose is reabsorbed to some degree, the fructose clearance being smaller than the creatinine clearance.

It would seem quite natural to suppose that the reabsorption of fructose and glucose goes on by closely related processes, and then the reabsorption of one might influence the reabsorption of the other. The reabsorption of glucose, however, must be supposed to take place more easily than that of fructose. It appears from the facts that glucose is practically completely reabsorbed when the concentration in plasma lies below the threshold, and that even a considerable fructosuria will cause no simultaneous glucosuria (WIERZUCHOWSKY, PIĘSKOW and OWSIANY 1931). Therefore, if the reabsorption of the two monohexoses takes place by the same mechanism it is at any rate necessary to suppose, that from a glucose-fructose mixture the kidneys will be most able to reabsorb the glucose. In that case it would be safe to expect a very considerable change in the fructose-creatinine clearance ratio when the glucose concentration of the plasma is raised.

From the experiments in this paper it appears that no great change is found in the fructose-creatinine clearance ratio when the glucose concentration of the blood is altered. So it must be concluded, that the reabsorptive mechanisms of glucose and fructose differ at least in regard to the process which is the principal determinant of the maximal velocity of the reabsorption.

The experiments concerning the influence of the diuresis on the fructose output show the reabsorption of the substance to be an active process. It is true that there seems to be a slight increase in the clearance ratio at the extremely large urine flows used in the cited experiment, but if the reabsorption was solely due to passive diffusion a far greater dependence on the urine flow would be found. In the first part of the experiment the difference in concentration between serum and bladder urine is about 700—800 mg per cent, in the second part it is only about 20 mg per cent. It is obvious that the results of the experiment cannot be explained under the terms of passive diffusion in a simple system, where the chief determinants of the diffusion are the difference in concentration and the time of contact.

The amount of fructose reabsorbed per minute must be supposed to depend upon the concentration of fructose in the part of the tubule where the process is going on. So the experiment shows in addition, that the reabsorption takes place in a rather proximal part of the tubule, where the concentration is as yet not influenced by the actual diuresis.

The experiments on the excretion of fructose at different concentrations in the blood also indicate, that the reabsorption of fructose is due to active processes, for the clearance ratio obviously depends upon the serum concentration. On the other hand we did not succeed in establishing a definite maximum for the amount of fructose which the tubule cells are able to convey per minute. This may be due to the fact that sufficiently high serum concentrations have not been investigated. Neither have we succeeded in finding a lower concentration of fructose in the urine than in the blood, even at concentrations of 3—4 mg per cent in the serum. This holds even if it is considered that at these low concentrations in the blood, the amounts of fructose found in the urine are a little too high owing to the blind, for which no correction has been made. A blind of 0.1 mg per min. will produce an error not exceeding 33 per cent.

The excretion of fructose differs essentially from that of several other substances which are actively reabsorbed from the tubules. Substances like glucose and sodium are practically completely removed from the filtrate by the kidneys, if the concentration in plasma is low, and at any rate in the case of glucose this holds true until the amount offered to the tubule cells is equal to their maximal capacity of reabsorption. The reabsorption of fructose, on the other hand, is highly insufficient even at very low concentrations.

### Summary.

The renal excretion of fructose is studied in dogs under different conditions. It is shown that the fructose is reabsorbed in the kidneys to some degree. The reabsorption is not influenced by alterations of the glucose concentration in the filtrate or by a considerable increase in the urine flow produced by the infusion of sulphate. On the other hand the excretion of fructose depends upon the concentration in the blood, a greater part of the filtered fructose being excreted at a higher plasma concentration.

It is concluded, that a part of the filtered fructose is actively reabsorbed in the proximal part of the tubules by a process, the faculty of which to convey the fructose is not influenced by the amount of glucose which is simultaneously to be reabsorbed.

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## The Exchange of Ions between Cells and Extracellular Fluid.

### I.

#### The Uptake of Potassium into the Chorion Membrane from the Hen's Egg.

By

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Received 31 May 1943.

Until recently it was very generally assumed that adult animal cells are impermeable to most ions and especially to kations, and this was accepted as an explanation of the very different compositions of intracellular and extracellular solutions respectively, although facts have been known for a long time which were, to say the least, very difficult to reconcile with the conception of impermeability. The demonstration by HEVESY and his collaborators by means of isotopes that a regular, even if slow, exchange both of anions and kations takes place in all the cell-types investigated (viz. erythrocytes, voluntary muscle, heart, liver, brain and yeast cells according to HEVESY and ATEN 1939, HEVESY and HAHN 1941, HEVESY and NIELSEN 1941) makes it necessary to thoroughly revise these conceptions and raises a series of new problems which it is proposed to discuss and to study experimentally in a small series of papers.

In the case of phosphate, which is as a rule much more concentrated within animal cells than outside, HAHN and HEVESY (1942) made it extremely probable that the concentration of free phosphate ions is in the steady state practically identical inside and outside cells, but is within the cells the first link in a system of phosphoric compounds which are constantly being

built up and degraded and for which the cell wall is truly impermeable.

The idea that a similar mechanism might be responsible for the large excess in potassium concentration inside most cells was of course tempting and was given some support by the discovery (HAHN and HEVESY 1941) that in certain cells (muscle, liver and others) a large fraction of the potassium present would exchange extremely slowly if at all with radioactive potassium added to the extracellular fluid. The difficulties in the way of such a conception are formidable, however. It is agreed on all sides, and has been abundantly confirmed by experiments with heavy water in the last few years, that almost all cells in animals and plants are freely permeable to water, and this involves a definite relation between inside and outside total osmotic pressure.

In most mature plant cells we have a rigid, but freely permeable, wall of cellulose or some allied substance outside the protoplast proper, which latter is usually present as a continuous layer covering the inside of the cellulose wall and enclosing in its turn a large vacuole containing the cell sap. From giant cells like those of many Characeae the cell sap can be isolated and analysed and is shown to be a solution mainly of free inorganic ions with a small admixture of organic substances. (COLLANDER 1930, 36, 39). Owing to the mechanical support rendered by the cellulose wall the total osmotic pressure can be, and usually is, much higher than in the outside fluid. The hydrostatic pressure inside the cells reaches such a height that the movement of water outwards is equal to the osmotic flow in the opposite direction. This makes the cells very rigid and it is not an infrequent occurrence that the internal pressure bursts open the cell. As in these cells the protoplast is slightly permeable to both anions and cations, while the inside concentration especially of potassium is maintained at a level up to several thousand times the outside, it seems necessary to assume an active transport (an "adenoid" activity OVERTON (1896), COLLANDER) transferring ions and above all  $K^+$  from the low outside to the high inside concentration. LUNDEGÅRDH (1940) attempted to picture a mechanism by which such transport can take place, but I fear it must be admitted that the picture is incomplete and highly speculative. I shall return to it at the end of this paper.

When plant cells are exposed to solutions of non penetrating or very slowly penetrating substances and of higher osmotic

pressure than that of the cell sap they contract by losing water to the outside until osmotic equilibrium is reached, and it is seen that the protoplast itself offers no significant mechanical resistance against changes in cell volume.

In almost all animal cells there is no mechanically resistant cell wall and no large vacuole containing cell sap, but the cell water contains osmotically active substances, and a practically perfect osmotic equilibrium with the surrounding solution is a necessary consequence of the free permeability of the surface layer for water. This is what has been demonstrated again and again for red blood cells, in which volume changes are induced by concentration alterations of the surrounding solution and are found to correspond quantitatively to such alterations. An osmotic pressure equal to that of the surrounding solution, that is corresponding in warmblooded animals to about 0.9 % NaCl (= 154 millimoles (mM) per liter), cannot possibly be obtained from organic slightly dissociated or complex potassium compounds, and the two significant facts viz.: The presence of a high potassium concentration and the existence of an osmotic pressure corresponding to that of the bathing solution are only compatible with the existence of the whole or a large fraction of the potassium as free K<sup>+</sup>. As shown by HEVESY the K<sup>+</sup> can diffuse slowly through the protoplasmic surface and must largely become lost in the course of a few days, unless it is replaced by some active transport from the low concentration outside towards the high concentration inside.

We have to account further for the fact that not only is K<sup>+</sup> retained inside the cells, but at the same time Na<sup>+</sup> is kept out, although it has been shown by experiments with radioactive isotopes to be able to diffuse slowly through the protoplasmic surface (HAHN and HEVESY 1941). This again appears to require some mechanism for active transport of sodium ions in the opposite direction.

The experiments to be described in the present and following papers were undertaken to establish the fact of such active transport and to obtain information, if possible, regarding its mechanism and regulation.

I had hoped to be able to study tissue cultures, but sufficient material could not possibly be obtained from these, and on the advice of dr. ALBERT FISCHER I selected for the preliminary experiments the Chorion membrane from hens eggs incubated 9

days. At this stage the embryo, the amniotic fluid and the yolk are surrounded by a double membrane consisting of an outer (ectodermal) and an inner (entodermal) epithelial layer and between the two there is a loose network, richly vascularized, of primitive connective tissue cells. Between these cells there exists a continuous extracellular space. I have spread such membranes without undue stretching, cut out discs of known area with a corkborer and weighed these in the fresh state and after drying to constant weight. The moist weight is quite variable (10—15 mg/cm<sup>2</sup> in my determinations on membranes about 1 hour after removal from the egg), while the two samples dried gave respectively 0.69 and 0.67 mg/cm<sup>2</sup>.

Dr. ALBERT FISCHER very kindly prepared for me some sections (5  $\mu$  thick) of the chorion and photographed selected parts at a magnification of 120. On these photos I have made a few measurements of the membrane thickness and counted the stained nuclei. These measurements are very rough, involving only a membrane surface area of 4,300  $\mu^2$  and a total count of 581 nuclei, but sufficient to give an approximate idea of the relations.

The average thickness as measured works out as 156  $\mu$  in satisfactory agreement with the fresh weight determinations of 10—15 mg cm<sup>2</sup> and corresponding to an area of 65 cm<sup>2</sup> per g fresh weight. Of the measured thickness the surface epithelium on one side makes up about 9  $\mu$  and on the other only about 1  $\mu$  or a total of 6.5 % of the whole. The total number of nuclei is about 10<sup>9</sup> per g fresh weight of which I found 40 % in the thick epithelium and 9 in the thin.

Of the 51 % internal nuclei I found 43 % in the connective tissue and 8 % in a blood vessel.

The 4 epithelial surfaces exposed to the bathing solution have, as a result of the irregularities, probably an aggregate area per sq. cm of 5 cm<sup>2</sup> or 320 cm<sup>2</sup>/g fresh weight.

In 1 g tissue the volume between the two epithelial layers is about 0.94 ml, but the cells make up only about 0.3 ml the rest being extracellular, as found in experiments given below.

If we take the  $4 \times 10^8$  cells occupying this volume to be spheres the radius of each works out at 5.5  $\mu$  and the total surface of all at 1,600 cm<sup>2</sup>. The cells are very far from being spheres, and 3,200 cm<sup>2</sup> is a more probable estimate for their total surface. This would make the epithelial surfaces just 10 % of the internal,

while the volumes are in the ratio  $\frac{65}{300}$ . It can be safely stated that the connective tissue cells constitute the preponderant element in the membrane, while the two layers of epithelium make up a fraction which is far from negligible.

The amniotic fluid normally bathing the membrane is a clear solution containing about 12.5 mg dry substance per ml of which 7 mg is inorganic. We have found it to contain reducing substances corresponding to 40—50 mg% glucose, 7 mM potassium and a total of 125—135 mM kations. These determinations were not made until the experiments were practically finished and these were carried out in normal Tyrode solution with a kation concentration about 150 mM.

### General Methods.

I have received the Chorion membranes (usually from 20 eggs) from dr. FISCHER's institute, where eggs at this stage are regularly opened for preparation of embryo extracts. They are removed aseptically, cut into pieces of some 20 sq. mm., to facilitate the access of the experimental solutions to the extracellular spaces between the epithelial membranes, and transferred to sugar and potassium free Tyrode solution in which I receive them on an average 1 hour after the preparation. They are then rinsed several times in the same solution and transferred to the experimental vessels. In this process the smallest pieces of tissue, which sink slowly, are removed.

The Tyrode solution normally used is made up as follows: 4 ml 20 % NaCl + 4 ml (0.5 % CaCl<sub>2</sub> with 0.25 % MgCl<sub>2</sub>) are diluted with redistilled water, 2 ml 5 % NaHCO<sub>3</sub> with 0.25 % NaH<sub>2</sub>PO<sub>4</sub>. H<sub>2</sub>O are added and the volume is made up to 100 ml. To this solution suitable volumes of isotonic KCl, isotonic glucose or saccharose or isotonic sodium thiocyanate can be added.

A series of preliminary experiments using different procedures and containers gave the result that the respiratory metabolism of the membranes thus treated and supplied with potassium and sugar would keep approximately constant for at least 5 hours at 38° C.

In the final series of experiments the membranes were distributed into 1—4 containers as shown in fig. 1 and centrifuged at 500—1,500 rev./m for 3 minutes. The supernatant fluid was sucked off and the containers weighed to obtain the moist

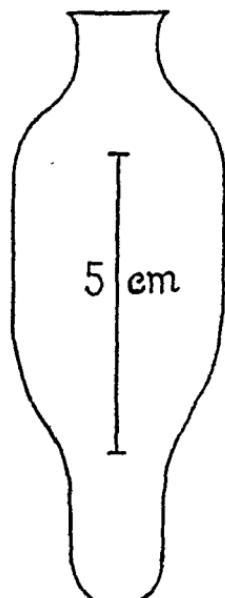


Fig. 1.

weight of the membranes in each. To one or more of the containers is then added a known volume of Tyrode solution containing either sodium thiocyanate or saccharose or both in suitable concentration to determine the extracellular space of the mass of membranes.

I give as an example the experiment made on Jan. 27 in which conditions were unusually complicated and the agreement unusually poor. The mass of membranes weighed at 10° 3.61 g. To this was added 3 ml of a Tyrode solution (A) containing 109 relative units of CNS and 109 of saccharose. (The actual concentrations were 8 mM thiocyanate and 78 mg% saccharose). After mixing for 25 minutes at 38° and centrifuging the relative concentrations in the supernatant fluid were determined at 11° as 60 and 56.5 respectively from which

$$\text{I calculate extracellular volumes of } \frac{300 - 180}{60} = 2.0 \text{ and } 2.32 \text{ ml respectively.}$$

Numerous experiments have shown that a complete mixture of the added fluid with the extracellular solution is obtained in 15 minutes. The specific gravity of the solution is 1.007, and the weight of the cells works out as 1.02 and 0.79 g respectively. A 2.00 ml sample was taken and replaced by 2.00 ml fresh solution and at 12° a fresh sample was drawn. This gave the relative concentrations 73 for thiocyanate and 61 for saccharose. Calculated from the results at 11° the concentrations should have been respectively 76 and 73. This difference is taken as indicating a contraction of the cells which are supposedly reduced from about 0.9 to about 0.65 g. At this point the supernatant fluid was sucked off and 4 ml solution B containing neither CNS nor saccharose added, mixed at room temperature for 15 minutes, centrifuged and sucked off. The weight of the mass of membranes now amounted to only 1.74 g. Again 2 ml of the solution B were added and the mixture left at 7° for 2.9 hours until 15°. Analysis of the fluid now gave relative concentrations of CNS 11 and of saccharose 11.5 and the weight of the mass of membranes was 1.72 g. 2 ml of solution A were added and after 0.9 hours the concentrations were found to be respectively 68 and 65.5 from which I find extracellular volumes of 1.23 and 1.28 respectively, corresponding to cellular weights of 0.43 and 0.41 g.

It is a perfectly regular occurrence that the mass of membranes contracts during the experiments, and dr. FISCHER informs me that the same happens in tissue cultures cut loose from the substrate. He finds an initial rather large contraction and a slow after effect, the latter corresponding to my observations. In this particular case the contraction reduced not only the extracellular space, but apparently also the cells themselves, but in several other experiments similar calculations have shown only an extracellular contraction, while the cells retained their volume. This latter seems to be the case when conditions are more nearly physiological (i. e. a suitable concentration of potassium, glucose and oxygen and the normal temperature of 38°) than in the present instance, but even in exactly parallel samples small but significant differences may be observed. Figures indicating a swelling of the cells by potassium absorption have not been met with however.

In the experiments proper the changes in composition of the total extracellular fluid were followed by analyses of potassium, total kations and usually glucose. Again I select as an example the experiment of Jan. 27 carried out on 1 sample only. The calculations are presented in the form of table 1.

The observed concentration changes depend only upon the analytic accuracy discussed in the subsequent section. There can be no doubt therefore that potassium is removed from the extracellular fluid into the cells at 38° with access to glucose and moves in the opposite direction during the intervening period at low temperature without sugar. The figures representing the quantities so transferred depend also on the relative volumes of cells and extracellular fluid and are therefore liable to somewhat larger errors.

When potassium ions are transported into a cell they must either be accompanied by an equivalent number of anions or be exchanged with an equivalent number of other kations. In the first case a corresponding volume of water must be osmotically attracted and a swelling take place which leaves the osmotic concentration on either side unchanged. In the second case both the cell volume and also the osmotic concentration on both sides will remain unchanged.

In the experiment reported in table 1 a significant increase in kation concentration of the extracellular solution is observed in periods 1, 2 and 4, while the changes in the remaining periods 3 and 5 are within the limits of error. This requires an explanation and two possibilities present themselves, viz. An intracellular increase in organic molecules small enough to be osmotically active, but of such nature that they cannot diffuse out, or a liberation of inorganic substances previously combined in such a way as to be osmotically inactive. No material is available to show definitely that any of these possibilities offers the true explanation.

In certain experiments I have endeavoured to trace the potassium further inside the cells. Determinations were made according to two different methods. In one a suitable sample (about 400 mg) of the tissue available at the end of an experiment was weighed into a platinum boat, dried completely and the dry substance determined by weighing. The boat was then placed in a quartz tube in a small electric oven and ignited in a current of air. When only a small amount of carbon was left a small drop of molar hydrochlorid acid was added and evaporated off before the ignition was completed, so as to convert carbonates to chlorides, as I have found it impossible to obtain carbonates of constant weight by ignition. Finally the ash was weighed to 0.01 mg and dissolved in 2 ml distilled water. On this solution potassium was determined. Taking the amount of extracellular water and its potassium content as known the cellular concentration could be figured out.

In the second method a large sample of tissue (5—8 g) was put into liquid air and finely pulverized. The frozen powder was transferred to a cellophane bag for ultrafiltration in the centrifuge according to REHBERG (1943), and the filtration carried out at a temperature of

Table 1.

Jan. 27. Chorion membranes. Fresh weight 3.04 g.  
 Solution A: Tyrode with 13.6 mM K, 84 mg% glucose, 78 mg% saccharose and 8 mM NaCNS.  
 Solution B: Tyrode without K, sugars or thiocyanate.  
 Experiment first at 38° with potassium, then at 7° without and finally at 38° with K<sup>+</sup>.

	Vol.	P o t a s s i u m			K a t i o n s			G l u c o s e		
		Cone. mM/l	Quantity μE	Change μE	Cone. mM/l	Quantity μE	Change μE	Quantity mg	mg	Used up
10 <sup>18</sup> Extracellular	2.1	0.2	0.4		148.5	312		0	2.52	
Added Sol. A	3.0	13.6	+ 40.5		160.5	452		84	2.52	
Total, rotated at 38°	5.1	(8.0)	- 40.9		(149.5)	764		(49.5)	2.19	
11 <sup>18</sup> Sample	3.3	16.8	- 24.1		163.6	784		43		0.86
Withdrewn			- 6.6			- 307			- 0.86	
11 <sup>20</sup> Added A	2.0	13.6	+ 27.0		150.5	+ 301		84	+ 1.68	
Total, rotated at 38°	5.1	(7.8)	- 37.2		(152.5)	778		(59)	3.01	
12 <sup>18</sup> Sucked off	5.3	27.0	- 10.2		155	791		50	2.55	
12 <sup>18</sup> Extracellular	1.1	(5.8)	5.8		(155)	171		(50)		
Added Sol. B	4.0	0.05	0.2		(152)	608				
Total, mixed at 20°	5.1	(1.2)	6.0		(152.5)	779				
12 <sup>18</sup> Sucked off			7.6			782			3	
12 <sup>18</sup> Extracellular	1.1	1.5	1.7		153	168				
Added Sol. B	2.0	0.05	0.1		152	+ 304				
Total, kept at 7°	3.1	(0.6)	1.8		(152)	472				
15° Sucked off		3.0	12.1	+ 10.3	155	481			9	
15 <sup>18</sup> Extracellular	1.1	3.9	4.3		155	171			2	
Added Sol. A	2.0	13.5	+ 27.0		150.6	301			0.02	
Total, rotated at 38°	3.1	(10.1)	- 31.3		(152)	472			84	1.68
15 <sup>18</sup> Final sample	6.4	19.8	- 11.6		153.5	476		40	1.70	0.53

0° ( $\pm 0.5^\circ$ ) to guard against any breaking up by enzymes of organic compounds. On the ultrafiltrate I have determined specific gravity, kations and in a few cases total osmotic pressure directly, and finally a sample of 400  $\mu\text{l}$  has been evaporated down to dryness in a platinum boat, weighed, ashed (with a small drop of n HCl), again weighed and dissolved in 2 ml water. On this solution the potassium determinations were made and the results distributed on cells and extracellular fluid as above.

As an example the experiment of Febr. 17 is reproduced. The membranes from about 60 eggs were distributed nearly equally into 4 containers and exposed to potassium and sugar free Tyrode solution. To two of these NaCNS was added at once, to two others about an hour later, and in all the volume of extracellular fluid was determined from the observed dilution of the thiocyanate. The specific gravity of the extracellular solution was 1.007<sub>3</sub> and the corresponding weights are given in table 2. In the two hour experimental period a total of 33 microequivalents ( $\mu\text{E}$ ) of K<sup>+</sup> was given off from the cells, while the kation concentration of the extracellular fluid remained almost unaltered (a total increase of less than 10  $\mu\text{E}$ ). The cells were finally in approximate "equilibrium" with a fluid containing on an average 1.65 mM K and 157 mM kations.

Table 2.

Containers	A	B	C	D	Total
Weight 11 <sup>50</sup> . . . . . g	3.381	3.015	2.915	3.128	
Weight of extracell. fluid . . g		2.365		2.47	
Weight of cells-calculated . . g		0.65		0.66	
Weight 12 <sup>48</sup> . . . . . g	2.342		1.944		
Weight of extracell. fluid . . g	1.58		1.52		
Weight of cells-calculated . . g	0.76		0.425		2.495 g
Final weight 13 <sup>40</sup> . . . . . g	2.134	1.840	1.721	2.171	7.866 g
Cell weight per cent of final . .	35.6	35.3	24.7	31.2	31.7 %
Final weight extracellular . . g	1.474	1.190	1.296	1.51	5.47 g

Of the final mass of membranes a sample of 384 mg was dried and found to contain 28.5 mg dry substance and consequently 355.5 mg water. The dry substance gave 3.91 mg ash. This ash was dissolved in distilled water and determinations of K<sup>+</sup> and total kations made. Assuming the ash to be dissolved in the water originally present in the tissue sample we should have 359.4 mg solution with a volume of 357  $\mu\text{l}$ . For this solution a K content of 10.6  $\mu\text{E}$  and a total kation content of 56  $\mu\text{E}$  was calculated. According to table 2 the weight of the sample can be distributed with 31.7 per cent or 122 mg on the cells and the rest 262 mg on extracellular fluid. The corresponding water content will be approximately 95 mg in the cells and 260.5 in

the extracellular fluid. The extracellular fluid of the composition given above would thus account for  $0.43 \mu\text{E}$   $\text{K}^+$  and  $41 \mu\text{E}$  kations and for the cells would be left  $10.2 \mu\text{E}$   $\text{K}^+$  and  $15 \mu\text{E}$  kations giving concentrations of  $107 \text{ mM}$   $\text{K}^+$  and  $158 \text{ mM}$  kations.

The ultrafiltrate from the mass of tissue left after the experiment showed the following concentrations: Thiocyanate 37.5 relative units, kations  $159 \text{ mM}$ , total osmotic concentration  $160 \text{ mM}$  and  $\text{K}^+ 15 \text{ mM}$ . The final extracellular concentration of thiocyanate being 51.5 it is calculated that 27.5 % the filtrate comes from the cells and 72.5 % from the extracellular fluid in very good agreement with the result calculated from the relative weights (water  $\frac{95}{355.5} = 26.7 \%$  from cells).

The cellular  $\text{K}^+$  concentration is found from

$$\begin{aligned} 27.5 x + 72.5 \cdot 1.65 &= 1,500 \\ x &= \frac{1380}{27.5} = 50. \end{aligned}$$

The large discrepancy between the  $\text{K}^+$  determinations on the ash and the ultrafiltrate respectively must be due to a gross error on the former, not noticed at the time and therefore not controlled by a repetition of the determination, as other similar determinations have shown that in this case the concentration calculated from the ultrafiltrate must be much nearer the truth. Two ashings were carried out on the ultrafiltrate giving, with very good agreement,  $14.1 \text{ mg}$  dry substance and  $9.13 \text{ mg}$  ash per ml. The sum of  $\text{Na}^+$  and  $\text{K}^+$  found and calculated as chlorides would give a weight of  $9.5 \text{ mg}$ .

#### *The analytical procedures employed.*

Potassium is determined by titration as iodoplatinate, mainly according to the directions from the Carlsberg laboratory (NORBERG 1938). Samples of the solutions to be determined are measured off into quartz micro test tubes by Carlsberg pipettes of suitable capacity from  $399 \mu\text{l}$  downwards to  $120$  according to the concentration expected. To the smaller samples a suitable amount of pure  $\text{NaCl}$  is added so that the total kation concentration is about equal in all samples. This was found to give the most uniform results. The samples are evaporated down and ignited in an electric oven at  $450^\circ \text{C}$ , and when most of the carbon has disappeared after about  $1\frac{1}{2}$  hour a small drop of normal  $\text{HCl}$  is added, evaporated off and the ignition completed when the samples are again dry. Special experiments have shown that no significant amount of ash is lost by evaporation during a period of several hours in the oven. After cooling  $2 \text{ ml}$  distilled water is added to each sample, and after a suitable time for solution  $1.5 \text{ ml}$  are drawn off and transferred to centrifuge vessels with a pocket at the tip holding some  $10-20 \mu\text{l}$ .  $0.1 \text{ ml}$   $2\% \text{ H}_2\text{PtCl}_6 \cdot 6\text{H}_2\text{O}$  is added to each and the content evaporated to complete dryness in a waterbath at  $100^\circ$ . The dry residue is washed thrice in the centrifuge with  $2, 1$  and  $1 \text{ ml}$  absolute alcohol.

The few drops of alcohol left over from the final washing are removed by evaporation at 60°, and to each vessel is added 0.5 ml of a buffer solution (pH 6.98) and 0.1 ml 3 % potassium iodide. According to NORBERG the colour of potassium iodoplatinate normally takes half an hour to develop, but we have found it to require more time and variations appear to be rather frequent. After several trials of a photometric estimation I have preferred a titration with thiosulphate which, however, also leaves much to be desired. Washing with the buffer solution I transfer the samples to numbered test tubes all having the same diameter and dilute them with the same buffer solution to approximately the same colour intensity so that 1  $\mu$ E of K<sup>+</sup> is present in about 2 ml. By the titration the colour changes from wine red to greenish yellow, and the end point is determined by comparison against a white background with a test tube charged with brom-thymol-blue in a phosphate buffer at pH 6.25. As the shade of colour of the dye solution cannot be made identical with the fluid titrated the analyst must select the concentration and pH which he finds most convenient. I have titrated quantities of 2—4  $\mu$ E with 7 mM thiosulphate in a 2 ml burette and smaller quantities usually with 3.5 mM. In almost every case one to three known solutions of potassium chloride are titrated along with the samples from an experiment and the results obtained on these, minus the titration value of a blank, used to calculate the unknowns. The titration value of 1  $\mu$ E of K<sup>+</sup> usually varies between 0.138 and 1.44 ml. The final accuracy obtainable is of the order  $\pm 5\%$ , but it cannot be denied that larger deviations are by no means rare and have most often been found inexplicable.

*The sum of kations* is determined electrolytically according to the method of ADAIR and KEYS in the modification recently described from this laboratory by HOLM-JENSEN (1943). The kations are transferred by amalgamation with mercury to a known quantity of acid (1 ml, 20 mM) and titrated with 0.2 n NaOH using methyl red as indicator. Determinations are made on 0.1 ml solution of the normal plasma concentration (about 150 mM). The accuracy is about 0.1  $\mu$ E. The alkali metals are transferred quantitatively, calcium to the extent of 90—100 % and magnesium only between 20 and 50 %.

*Glucose* is determined according to HAGEDORN (1935). The accuracy is about 2 mg %.

*Saccharose.* Two identical samples are taken. One is directly precipitated and a glucose determination made. To the other is added 1 ml  $\frac{n}{10}$  HCl and the mixture is boiled for 10 minutes to invert the saccharose. After cooling it is neutralized with 1 ml  $\frac{n}{10}$  NaOH and precipitated as above. The difference between the two titrations is calculated as saccharose. Relative values only are needed for estimating the extracellular space and with the concentrations used the accuracy is about 2 per cent.

*Thiocyanate.* A sample of suitable volume (0.1—0.2 ml) is precipitated with 1 ml 10 % trichloroacetic acid. After standing a few hours

it is centrifuged. 0.8 ml are pipetted off with a Carlsherg pipette and transferred to another test tube to which is added 1 ml water and 0.5 ml Crandalls reagent (quoted from Lavietes et al.) giving a reddish colour proportional to the CNS concentration. The depth of colour is determined by photoelectric colorimetry. The accuracy is about 1 per cent.

The total osmotic pressure has been determined in a few cases according to the thermoelectric method of BALDES (1934) as modified in this laboratory (KROGH 1939, p. 211), and in a single instance according to the method of URSPRUNG and BLUM (1930) by measuring the change in length of columns of solution in capillary tubes, which has also been modified here so as to give an increased accuracy. The BALDES method is accurate to 1 mM. The modified URSPRUNG and BLUM method is accurate to about 3 mM.

### Results.

A presentation of the results is conveniently started by comparing the experiment of Febr. 17, given in detail above, with a similar one of March 10 in which the membranes after the usual washings were treated for about 2 hours at 38° with Tyrode solution having at the start a concentration of about 13 mM potassium which was reduced during the treatment to an average of 5.4 mM. The kation concentration in the extracellular fluid remained practically constant throughout at 151 mM.

To the measured fall in extracellular  $K^+$  corresponded an uptake into the membranes of  $140 \mu E$  or  $16 \mu E$  per g wet weight. The total quantity found in the tissue at the end of the experiment amounted to  $47 \mu E/g$  against  $27 \mu E$  in the exp. of Febr. 17 in which about  $4 \mu E$  per g had been lost to the surrounding solution.

According to the thiocyanate determinations 100 g membranes should contain 57.4 g extracellular fluid and 42.6 g cells containing respectively 56.8 and 33.2 g water.

The distribution of the  $K^+$  on extracellular (5.4 mM) and intracellular water gives for the latter a  $K^+$  concentration of 132 mM. Analysis of the ultrafiltrate gave per g water  $41.5 \mu E$   $K^+$  and a corresponding distribution gives 116 mM for the  $K^+$  concentration of the cell water.

The difference between 132 and 116 exceeds the analytical error to be expected and might be due to the presence in the cells of organically bound potassium liberated by the ashing, but it may also be ascribed to a slight preponderance of extracel-

lular fluid in the ultrafiltrate, a possibility which cannot be ruled out. Special experiments have failed to reveal the presence of any kations in osmotically inactive combination, but are not sufficient to disprove it. In any case the two experiments, taken together with a couple of similar but less complete ones, show that the chorion cells contain the ions of  $K^+$  and  $Na^+$  in the free state, that these, together with the undetermined anions, make up a very large fraction of the total osmotic pressure (probably more than 95 %), that the diffusion processes going on all the time tend to equalize the concentration of each of these on the two sides of the protoplasmic membrane of each cell, but that the diffusion is balanced by an active process, requiring energy, which transfers potassium ions from the extracellular to the intracellular solution and sodium ions in the opposite direction.

My experiments cannot give any information concerning the mechanism of this active transfer, but a few points concerning the conditions have been more or less established.

In one experiment one sample of membrane was washed twice with a Tyrode solution containing neither calcium nor potassium, another with calcium (1.8 mM), but without potassium. They were thereupon rotated for 4 hours at 38°, one without, the other with calcium, and both without potassium and sugar. The first lost 5.6  $\mu E$   $K^+$  per g fresh weight, the second 5.9 and the extracellular  $K^+$  concentration rose to 0.7 mM.

When glucose was added to a concentration of about 100 mg% and KCl to a concentration of 3.9 mM,  $K^+$  was rapidly absorbed, viz. 18  $\mu M$  in 85 min in the Ca-free and 21 with Ca reducing the concentration in both cases to about 0.8  $\mu E$ . A renewed addition of sugar and KCl resulted in a further uptake of 7  $\mu E$  without and 5.5  $\mu E$  with calcium in 2 hours giving final concentrations of 2.6 mM and 3.2 mM respectively. The amount of sugar used up was slightly less without  $Ca^{++}$  than with. Calcium concentrations were not determined and it is possible that the tissue may have contained enough to compensate the loss, but so far as it goes the experiment does not indicate any influence of calcium either on the loss of potassium by diffusion or on its active absorption.

The chorion membranes at 38° use up glucose at a fairly rapid rate corresponding usually to from  $1/3$  to 1 mg/hour per g fresh weight or 7—20 mg/h/g dry substance, while only about  $1/2$  or

less can be accounted for by the simultaneous  $O_2$  absorption. The uptake of potassium is rapidly reduced and may fall to 0 when glucose is removed, but I have found no relation between the rate of potassium uptake and the glucose consumption, and I suppose therefore that the energy involved in the uptake is probably only a fairly small fraction of the total metabolism. Even in the presence of glucose potassium is given off to a potassium free solution at  $38^\circ$ , but the concentration does not rise beyond 1 mM.

In one experiment lactic acid (100 mg%) was substituted for glucose and a rapid uptake of  $K^+$  was observed, but no determination was made of the consumption of lactic acid.

At a low tp. ( $3^\circ$ — $7^\circ$ ) the membranes lose potassium at the rate of 1  $\mu E/g/h$  or even less, and the amount of glucose catabolized is reduced to less than 0.1 mg/g/h which is within the limits of experimental error.

An oxidative breakdown of glucose does not appear necessary for the potassium uptake, but the experimental results are not quite consistent, and the uptake is no doubt retarded by oxygen lack while sugar disappears at a greatly increased rate.

In one set of experiments the usual containers were filled with a nitrogen atmosphere containing 1.0 %  $O_2$  and 1.7 %  $CO_2$ . In this case no uptake of  $K^+$  was observed, and in one period even a slight loss to an outside concentration of about 5 mM and with glucose 250 mg % or lactic acid 109 mg %. The glucose consumption rose to 2.3 mg/hour per g initial weight. When oxygen (about 50 %) was added a rapid uptake took place and the sugar consumption was reduced to less than 1 mg.

In another set of determinations the atmosphere was varied between 2 and 4 % and finally 50 % were given. In this case there was a definite uptake at the low  $O_2$  concentrations, but higher with 4 % than with 2 and again increased with 50 %. The sugar consumption was high both in 2 % and in 4 % oxygen without any significant difference and fell to less than  $\frac{1}{2}$  in oxygen.

On account of the consumption the  $O_2$  pressure must have been definitely lower in the cells, but a little oxygen has been available all the time, and an experiment was therefore made with special small containers (8 ml), which could be completely filled with solutions saturated beforehand with nitrogen (containing about 1 %  $O_2$ ). The trace of  $O_2$  present (2  $\mu l$ ) is used up in a few

minutes and the conditions become completely anaerobic. In spite of this the cells took up much  $K^+$  from an initial concentration of 11.5 mM. In one container 3.3 g membranes absorbed 27  $\mu E$  in 1 hour, while in another the same quantity took up only 22  $\mu E$  in 1.9 hours. In the following period with oxygen the first sample absorbed a further 23  $\mu E$  in 45 m., the second only 7  $\mu E$ . It would appear that the second sample became damaged by two hours anaerobiosis. A high concentration of glucose was present in both cases and the usual large amount catabolized anaerobically.

### Discussion.

Even if it is not possible to form any consistent picture as to how the active transport of ions can be brought about certain conditions can be defined which appear necessary.

The ions to be transported must enter into a fairly stable and specific combination with organic molecules in the very surface of the protoplasmic membrane of each cell. Any unspecific reaction or structure would be unable to account for the different behaviour of the membrane towards the two not very dissimilar ions  $K^+$  and  $Na^+$ .

From the quantitative relations described above it is evident that the connective tissue cells must be active in the transport and there is no reason to doubt that the power is shared by the epithelial cells.

It is convenient, even if the true mechanism may be quite different, to visualize the boundary layer, surrounding each cell and representing the medium through which the exchange takes place, according to the model proposed by LUNDEGÅRDH and showing essential similarities to the Langmuir films. It is taken as made up of long micellæ or molecules placed at right angles to the surface. In a Langmuir film all the micellæ are of the same kind; but LUNDEGÅRDH considers the boundary layer of plant cells as a mosaic containing both indifferent micellæ and others spaced at certain intervals which have at one or both ends very definite affinities. For the plant cells studied by LUNDEGÅRDH it is only necessary to assume different micellæ with cation and anion binding powers, but in the animal cells here under consideration it is necessary to postulate much more specialized micellæ, capable of binding specifically  $K^+$  and  $Na^+$  respectively. The boundary film must not be looked upon as a

static structure. Just as in Langmuir films the single micellæ turn round occasionally, and an ion combined to one end, say at the outside, will at intervals be presented on the inside face and may be split off. Such a structure may account for the "diffusion" exchange of ions and may also assist in the "active transport", if energy and machinery are available inside to reduce or increase the force with which ions are bound.

In the cell boundary layers here considered the micellæ responsible for potassium uptake can be spaced at, comparatively speaking, very considerable distances apart.

In one experiment the rapid uptake of  $138 \mu\text{E}$  by 8.5 g cells in 108 minutes from a solution containing from 7 to 13 mM K was noted, corresponding to  $2.5 \times 10^{-3} \mu\text{E/g/second}$  or  $1.5 \times 10^{15}$  individual ions/g/second. Assuming the low figure given above of a total surface of  $3,500 \text{ cm}^2$  for 1 g cells, and assuming further the very infrequent uptake at each active point of 1 individual  $\text{K}^+$  ion per second, the total of  $4.3 \times 10^{11}$  ions/ $\text{cm}^2/\text{sec}$  can be accounted for if the points are evenly distributed at intervals of 150 Ångström ( $1 \text{ \AA} = 10^{-8} \text{ cm}$ ). It is probable that the points are much further apart, and this might help to explain the fact that at outside concentrations not much below 1 mM the active uptake cannot balance the diffusion.

The mechanisms for active ion transport here under discussion require some kind of regulation. The experiments show that  $\text{K}^+$  can be absorbed from dilute solutions down to 1 mM or less, and if such absorption went on, without inhibition of some kind, from the concentrations present in the tissue fluids (about 7 mM in the amnion fluid of the hens egg) the cells could not avoid swelling by the water osmotically attracted.

It is conceivable, although a priori rather unlikely, that the actual concentrations found in the organism represent a balance between diffusion and capacity for active transport. If so any change in the kation concentrations of the blood and extracellular fluid would upset the balance and cause considerable changes within the cells.

It appears possible that cell volumes (or surface areas) are regulated, that an active uptake of potassium ions is induced when the volume of a cell becomes reduced — e. g. by an increase in the osmotic concentration of the extracellular fluid. This would cause an osmotic attraction of water along with the potassium uptake until the original cell volume was restored. Con-

versely the swelling of a cell might stop the active uptake and allow potassium to diffuse out until the volume was again brought back to normal.

It appears possible finally that the ratio between  $K^+$  and  $Na^+$  in solution within the cell is involved in the regulation and that a change in this ratio sets the machinery in motion.

A choice between these possibilities requires further experimentation for which the chorion membranes are unsuitable, both because two different cell types are involved, but also and especially because cell volumes cannot be determined with any satisfactory accuracy. Experiments on red blood corpuscles are in progress to study these regulation problems and will be reported on in a subsequent publication.

### Summary.

The fact that living cells both in animals and plants are generally permeable both to kations and anions makes it necessary to investigate the mechanisms by which the large differences in concentration of single ions inside and outside cells are maintained.

As a consequence of the general and free permeability for water the osmotic pressures inside and outside animal cells must be identical, and this involves the presence of at least a large fraction of the ions in the free state, because no other substances are available to reach such high concentrations.

Methods are described to study the transfer of ions between cells and extracellular fluid in isolated chorion membranes from the hens egg, to prepare ultrafiltrates from these membranes and to distribute the ions determined on cells and extracellular solution.

The chorion membranes are made up of two layers of epithelium and a loose network of primitive connective tissue cells with large extracellular spaces. Approximate measurements of numbers and dimensions of the separate cell types are presented.

It is found that prolonged washing with potassium free Tyrode solution will reduce the  $K^+$  content of the cells to about half the normal (50 millimoles), the  $K^+$  being replaced by  $Na^+$ , while  $K^+$  will be absorbed by the cells from the outside solution containing at the start 15 to 3 mM  $K^+$  down to concentrations

of 1 millimole or less, raising the concentration of the ultrafiltrate from the cells above 100 millimoles. Simultaneously  $\text{Na}^+$  is removed from the cells against the outside concentration of about 150 mM.

The two ions together with the undetermined anions make up a very large fraction of the total osmotic pressure of the solution in each cell.

The active transfer of ions in both directions requires energy which appears to be provided mainly by non oxidative breakdown of carbohydrate and can go on at least for some time in the absence of free oxygen.

At low temperature ( $3-7^\circ$ ) the active transfer is reduced or abolished and the diffusion tends to equalize the concentration of each ion across the protoplasmic boundary film.

It is pointed out in the discussion that specific structures and mechanisms involving a binding of the ions of elements in the boundary film must be assumed to account for the active transfer. The mosaic membrane model proposed by LUNDEGÅRDH is discussed and it is shown that the "points" responsible for potassium uptake are probably spaced at comparatively large distances apart.

The possible mechanisms for regulating the transfer are briefly discussed.

The author is greatly indebted to dr. ALBERT FISCHER of the Carlsberg Foundation Biological Institute for his constant help and advice and also to dr. I. HOLM-JENSEN and Miss A-L. LINDBERG of this laboratory who carried out with great care much of the analytical work involved.

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## On the Partition of Certain Amino Acids between Blood and Tissues.

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Received 4 June 1943.

### Introduction.

In 1913 VAN SLYKE found that protein-free tissue extracts showed a higher content of amino N than did deproteinized plasma. Since the amino N of these extracts was only slightly augmented by acid hydrolysis, he concluded that the amino N came from amino acids and only to a very slight extent from peptides. This would mean that the living cells contained a 4—10 times higher concentration of free amino acids than the surrounding medium, and this view has since been commonly held (compare LUCK (1928)). No explanation for this peculiarity has as yet been given.

In a previous paper (USSING (1943)) it was shown, that the high amino N content of red corpuscles originated for the greater part from glutathione. Indeed the amount of real amino acid was found to be the same in the water-phases of plasma and corpuscles.

In most tissues glutathione is present in abundance (255 mg % in liver, 169 mg % in kidney, 65 mg % in muscle according to FUJITA and NUMATA 1938), and as glutathione gives nearly double the calculated amount of N<sub>2</sub> in the VAN SLYKE apparatus (HOPKINS (1929)) it will be seen that an appreciable correction on the amino N of the tissues will have to be introduced. The fact that glutathione gives more than the calculated amount of amino N already before hydrolysis may explain why VAN SLYKE (l. c.) found relatively little increase in amino N on hydrolysis of the tissue extracts.

From the above considerations it is seen that VAN SLYKE determinations are unsatisfactory when the amount of real amino acids in the tissues is to be determined. Moreover it is not altogether certain that all amino acids behave in the same way. The safest way to investigate if free amino acids are concentrated in the tissues would evidently be to determine a suitable number of single amino acids in plasma and tissues. No such investigation has hitherto been made, but some information may be found scattered in the literature:

*Arginine*: ARNOLD and LUCK (1933), using the arginase-xanthidrole method, found that mammalian muscle contained 1—12 mg % arginine, corresponding to less than 1 mg % amino N; in liver small but fluctuating amounts were found. No figures were given because some arginine might have been split by the liver arginase. ABDERHALDEN (1913) isolated arginine from deproteinized blood, but the concentration of this amino acid in the blood was not determined.

*Histidine*: In an investigation of carnosine in mammalian muscle KAPPELER-ADLER and HAAS (1934) mentions that free histidine is absent in the mammals. On the other hand 88 mg % histidine was found in carp muscle.

*Cystine + Cysteine*: Using a colour reaction with dimethyl-p-phenylenediamine which is claimed to determine cystine and cysteine even in the presence of glutathione, FUJITA and NUMATA (1938/39) determined these amino acids in several tissues. In all cases the amount was insignificant compared with the amount of glutathione. In the tissues the amount of the amino acids in question varied between 0.8 and 13 mg % compared with 0.8 mg % in the blood.

*Glutamic acid*: COHEN (1939), using a method which involves the oxydation of glutamic acid to succinic acid with chloramine-T, and subsequent determination of the succinic acid formed by a succinic acid dehydrogenase method, found very high concentrations of glutamic acid in the tissues compared with the plasma. For instance guinea-pig kidney contained 94 mg %, whereas plasma contained 2.8 mg % glutamic acid. Glutathione does not disturb this analysis.

Glutamic acid is thus the only amino acid which has been with certainty shown to be concentrated in the tissues.

It is the intention in the present paper to examine some further amino acids as to their distribution between tissues and plasma.

The amino acids, which were selected for the investigation, were tyrosine, leucine and valine. Tyrosine was determined by a modified Millon reaction (ARNOW (1937)).

In the case of the lastmentioned two amino acids a micro modification of the method of FROMAGEOT and HEITZ (1939) was worked out. As the micro method is in some respects profoundly different from the macro method it will be described in some detail in the following.

## Experimental.

### a) Preparation of Tissue Extracts.

The experimental animals were killed by cutting the neck with a knife. The blood was sampled in a weighed vessel. Each of the organs, which were to be examined, was quickly excised and frozen in liquid air, weighed and pulverized, first in a cooled steel cylinder into which a steel piston fitted and then in a porcelain mortar. During the last pulverization liquid air was added and when the powder was sufficiently fine 4 volumes of 5 % trichloracetic acid was slowly poured in and mixed with the powder. When the resulting mass had melted, 5 volumes water (referred to the original amount of tissue) was added and when the liquid had attained room temperature it was filtered. 10 ml of filtrate corresponded to one g tissue.

The filtrate was concentrated under reduced pressure to about 20 ml and after addition of a few drops of dilute HCl it was extracted with ether in a continuous extractor.

After this the filtrate was concentrated and transferred to a graduated tube together with so much water that each ml corresponded to one g of tissue.

The samples so treated were used for the determinations of tyrosine as well as leucine + valine.

### b) Determination of Leucine + Valine.

The principle for these determinations is that when amino acids are deaminized and then oxidized with chromic acid in acetic acid, only leucine and valine will yield acetone. After distillation the amount of acetone formed is determined with the very sensitive and specific colour reaction with salicylic aldehyde.

In the original method (FROMAGEOT and HEITZ 1. c.) the proportion between leucine and valine could be evaluated because the hydroxy-isovaleric acid (originating from valine) is more rapidly oxidized to acetone than is hydroxy-isocaproic acid (originating from leucine). The different amount of acetone produced by short and prolonged oxidation is used for the calculation.

In the present case, the question being whether or not the tissues

do concentrate these amino acids, differential determinations are of minor importance. On the other hand the analyses have to be performed on small samples of tissue (1—4 g) and the original method would require about 20 times as much.

Most of the manipulations may be easily adjusted to small samples, simply by diminishing the vessels and the amounts of reagents.

The only serious difficulty is the quantitative distillation of acetone from a sample not exceeding 1.5 ml. After several vain attempts it was found that the distillation could be performed in a Conway unit. The alkaline solution of salicylic aldehyde is placed in the inner compartment and the acetone containing solution is introduced in the outer compartment, which, beforehand, has been provided with enough 30 % NaOH to make the oxidizing mixture distinctly alkaline.

It is necessary that the solution in the outer compartment is made alkaline because some of the alcohol which is used to keep the salicylic aldehyde in solution in the inner compartment diffuses out into the outer compartment. Here it would be oxidized to acetic aldehyde; this substance would in turn diffuse back to the inner compartment and here it would react with the salicylic aldehyde under the formation of a colour which is practically indistinguishable from the colour produced by acetone. According to FROMAGEOT and HEITZ (l. c.) the colour produced by acetic aldehyde is about 75 times weaker than that produced by acetone, but as the available amount of alcohol is some 200 mg and the amount of acetone is often less than 0.1 mg it is obvious that an intolerable blank would occur. In alkaline solution on the other hand, this formation of acetic aldehyde is brought to a complete standstill. The alkali has yet another important function: It keeps the acetic acid from distilling into the inner compartment. Such distillation should be omitted because the colour intensity is a function of the alkalinity.

### c) Procedure.

1) *Deamination:* 2 ml of the sample to be examined, containing less than 0.5 mg leucine + valine/ml is transferred to a pyrex tube together with 7 ml water and made neutral to lithmus; 0.25 ml n H<sub>2</sub>SO<sub>4</sub> is added. The tube is placed in a boiling water bath and 1.5 ml of 2.5 % NaNO<sub>2</sub> is slowly run in from a long pipette which reaches the bottom of the tube. The upper end of the pipette is provided with a rubber tube with a screw clamp to regulate the flow. This process should last 15 minutes. The tube is shaken every one minute. After the pipette has been emptied 1.5 ml 7.5 % urea solution is run in in precisely the same way to remove any excess of nitrite. The solution is transferred to a small pyrex distillation flask with detachable neck and the water is distilled off until only about one ml remains. It is then transferred by a pipette to a graduated tube which is filled up to the 2 ml mark with the washings from the distillation flask.

2) *Oxidation:* 1 ml samples of the deaminized filtrates are measured into small glass ampoules (50×10 mm) followed by 200 mm<sup>3</sup> glacial

acetic acid. The ampoules are cooled in ice and  $350 \text{ mm}^3$  10 % chromic acid (for solutions poor in organic matter  $200 \text{ mm}^3$ ) is quickly introduced into each ampoule upon which the ampoules are sealed. The ampoules are then heated in an electric oven at  $100^\circ$  for 4 hours. This time suffices according to FROMAGEOT and HEITZ (l. c.) to get the maximum formation of acetone from leucine. Valine requires considerably less time. After oxidation the ampoules are cooled, first in the air and then in an ice bath.

3) *Distillation and development of colour.* The acetone reagent is prepared by mixing in a small beaker 4 parts of 9 n KOH with 1 part of 6.7 vol. % salicylic aldehyde in 96 % alcohol. The mixture should be stirred well. 1 ml reagent is measured into the inner compartment of each Conway unit. It is advisable to grease the edge of the wall between the inner and outer compartment with a little vaseline to prevent the reagent from creeping over the wall. The cover is likewise greased with vaseline. Into the outer compartment 1 ml 30 % NaOH is measured.

The ampoules are opened one by one (care, high pressure!) and the contents of each is sucked into a Krogh's syringe pipette (KROGH 1935) — previously cooled with ice water — and delivered into the outer compartment of a Conway unit, which is then closed as quickly as possible. During delivery of the sample the cover should leave only the necessary space for the tip of the syringe pipette to be introduced into the outer compartment of the unit.

After 3 hours at room temperature ( $20-22^\circ$ ) the colour has reached its maximum strength. (In the original method the colour was developed by heating at  $50^\circ$  for one hour). The units are opened and the contents of each of the inner compartments is transferred with a pipette to a graduated tube. The inner compartment is washed repeatedly with water and the washings are combined with the main portion of colour. At last the tubes are filled to the 10 ml mark and well shaken.

The solutions are read in an electric photometer with a green filter. A leucine standard (0.5 mg leucine per analysis) and a blank are run together with the unknowns through all stages of analysis.

4) *Evaluation of the readings:* According to FROMAGEOT and MOURGE (1940) the yield of acetone from leucine is 48 % of the theoretical amount when heating with chromic acid for 4 hours is used. Under similar conditions valine yields 61 %.

As the theoretical yield is the same for both amino acids when calculated on a molar basis, it is seen that on using a leucine standard the amount of valine will be estimated about 20 % too high. This is of minor importance in the present case as the blood and the tissues must contain a mixture of leucine and valine.

Fig. 1, A shows the relation between colour, measured in arbitrary units and the amount of leucine. The photometerreadings are the difference between the galvanometer deflection produced by the blank, which is used as zeropoint, and the deflection produced by the solution to be tested, read on a logarithmic scale. This means that the units are proportional to the absolute amount of colour.

Fig. 1, B shows the same relation when 5.5 n KOH is used instead

Photometer  
reading

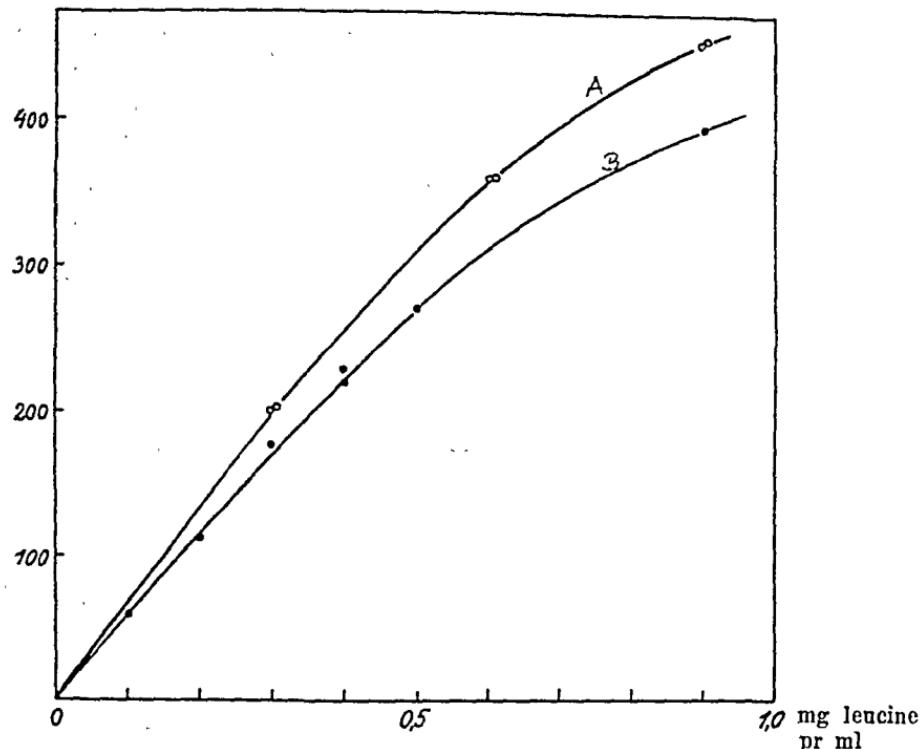


Fig. 1.

of 9 n KOH in the acetone reagent. It is seen that the photometer readings in both cases are proportional to the amount of leucine up to about 0.5 mg leucine per analysis. At higher concentrations this relation does not hold, which means that the colour does not follow Beers law of dilution at those concentrations.

The difference between the two curves may serve to demonstrate the influence of KOH concentration. The space does not allow a closer discussion of the coloured substance and the purely empirical nature of the method makes such discussion unnecessary. For literature concerning the colour reaction the reader is referred to FROMAGEOT and HEITZ (1. c.).

The difference between duplicate determinations seldom exceeds 5 %. In the dead space of the syringe pipette, which is used to transfer the analysis to the Conway unit, about 5 % of the analysis is lost, but as the standard solution is treated in the same way this source of error is insignificant. Similar considerations are valid for the small amount of fluid adhering to the walls of the ampoule.

### Results.

Determinations of leucine + valine and of tyrosine in blood and tissues were made on a series of guinea-pigs.

The determination of leucine + valine is described in the foregoing pages, tyrosine determinations were carried out according to ARNOW (1. c.). Tables 1 and 2 show the results. It is seen that none of the organs show any pronounced tendency to concentrate leucine and valine as compared with the level in the blood. (As shown by DANIELSSON (1933) and USSING (1. c.) the amino acids are evenly distributed between corpuscles and plasma and it is therefore unnecessary to separate the plasma for the determinations).

Table 1.  
mg Leucine + Valine per g Tissue.

	I	II	III	IV	V
Blood . . . . .	0.100		0.071	0.059	0.059
Liver . . . . .	0.118	0.078	0.074	0.082	0.076
Kidney . . . . .		0.087		0.077	0.103
Muscle . . . . .		0.068	0.060	0.039	0.079
Brain . . . . .					0.084

Table 2.

	I		II	
	Reading	mg tyrosine per g	Reading	mg tyrosine per g
Blood . . . . .	20	0.019	13	0.012
	20			
Liver . . . . .	18	0.018	14	0.018
	20			
Kidney . . . . .	36	0.038	35	0.038
	33			
Muscle . . . . .	17	0.017	12	0.011
	18			
Tyrosine, 0.15 mg .	160		160	

A larger material should be required to find out if the small difference found between blood and certain tissues in some of the guinea-pigs is significant, but it is already clear that these amino acids play no part in producing the considerable excess of amino N in the tissues.

Tyrosine seems also to be evenly distributed between blood and tissues. The kidneys are apparently an exception, their tyrosine

concentration being higher than that in the blood. It must be remembered, however, that peptides containing tyrosine, give the colour. (Other interfering substances like phenoles are removed by the ether extraction, which also removes  $\beta$ -hydroxy-butyric acid and other substances which might interfere with the determination of leucine and valine). The amounts of tyrosine found should be regarded as maximum values. The nature of the apparent excess of free tyrosine in the kidneys has yet to be examined.

Table 3.

	Colorimeter reading	mg leucine + valine/g tissue
Blood . . . . .	319 316	0.50
Liver . . . . .	270 303	0.45
Kidney . . . . .	219	0.35
Muscle . . . . .	115	0.18
Brain . . . . .	98 95	0.15
Leucine, 0.5 mg . . . . .	320 311	

For theoretical reasons it would perhaps be preferable to give the amino acid content in relation to the water phases of the organs. The water content amounts to about 80 % in blood, kidney and brain, 80—75 % in muscle and about 75 % in liver. It is obvious that the results would not be materially altered by a recalculation on water-basis.

It was found by LUCK (l. c.) that when amino acids or amino acid mixtures were fed to rats a considerable increase in the amino N of the liver took place. This increase was considerably higher than the increase of amino N in the blood. It was therefore necessary to examine whether an augmentation of the free leucine in the blood would produce a still greater increase in the leucine of the organs, especially the liver.

The following experiment was made. A guinea-pig ( $\text{♀}$ , 350 g) was narcotized with 0.6 g ethylurethane in 2 ml water subcutaneously. A glass cannula was laid in the right vena jugularis and 100 mg leucine in 10 ml 0.6 % NaCl was injected with a continuous injection apparatus during 45 minutes. After a further 5 minutes to allow mixing of the blood the animal was killed and

the leucine determinations on blood and organs were carried out at usual.

The results are presented in table 3.

It is seen that the leucine content of the blood is higher than the concentration in any of the organs. That the concentration in the muscles is much lower than in the liver and kidney must be due to a low permeability of the muscle cells towards leucine. The low concentration in the brain is more likely due to a low permeability of the capillaries of the brain towards leucine just as they are only slightly permeable to ions and sugar.

This experiment seems to speak in favour of the view that leucine is distributed between organs and blood by simple diffusion; but a real proof of this view cannot be obtained by this sort of experiment.

### Discussion.

It has been shown that the monamino monocarbonic acids leucine, valine and tyrosine are almost evenly distributed between blood and tissues. A quite even distribution may not be expected in all cases. First a certain breakdown of proteins to amino acids may take place from the killing of the animal to the moment when the cooling has stopped all enzymatic activity. Secondly the slow diffusion of for instance leucine, which has been found above for certain organs like muscle and brain, will have the effect that fluctuations of the amino acid level in the blood will only be visible in the amino acid level of the organs after some time. Thirdly the liver and perhaps other organs have a function in the regulation of the amino of the amino acid level of the organism. By deamination the liver may lower the level, while by breakdown of protein the level may be raised.

It is improbable that the other monamino monocarbonic acids should behave otherwise than the three examined here, with one possible exception: LUCK (1. c.) found that while feeding of alanine and other amino acids produced only slight increase in the amino N of the liver, glycine produced a considerable increase which lasted for hours. It would be of interest to know if the substance giving the increase in amino N is really glycine or possibly some derivative produced in the liver.

As mentioned in the introduction there is some evidence that the bases, arginine and histidine are not concentrated to any extent in the tissues.

The only amino acid which is known to be highly concentrated in the tissues is glutamic acid (COHEN (l. c.)). It may be of interest in this connection that glutamic acid and aspartic acid were found to diffuse very slowly if at all through the red corpuscle membrane (USSING (1943)). If the membranes of other cells were supposed to be relatively permeable to most amino acid but only little permeable to glutamic acid, then only this amino acid would be concentrated even if the cells possessed a general mechanism for the transport of amino acids into the cells. The low leucine content of the muscle, which has evidently a low permeability to leucine, shows however, that if a general mechanism of amino acid transport does exist it is not used to any high extent.

From the above it follows that amino N determinations on protein free tissue extracts say nothing about the amount of true free amino acids. Most of the amino N comes from glutathione and possibly other low peptides; in some animals, but not in the guinea-pig, carnosine plays an important rôle especially in the muscle (compare VIGNEAUD and BEHRENS 1939).

Table 4.  
Amino N in mg %.

	Liver	Muscle	Kidney	Plasma
Total ca. <sup>1</sup> . . . . .	45	45		5
Glutathione ca. <sup>2</sup> . . . . .	30	10	20	0
Glutamic acid . . . . .	5	16 <sup>a</sup>	9	0.3
Arginine . . . . .	+	< 1	+	+
Histidine . . . . .	(—)	(—)	(—)	(—)
Cystine + Cysteine ca. . . . .	0.7	0.2	0.1	0.1
Tyrosine ca. . . . .	0.12	0.11	0.25	0.12
Leucine + Valine ca. . . . .	0.8	0.6	0.9	0.7

<sup>1</sup> van Slyke amino N. — <sup>2</sup> Heart muscle.

Another implication is that if phosphoric acid esters or similar compounds of amino acids play a part in protein synthesis or amino acid transport (compare KJERULF-JENSEN 1942) they are only present in low concentrations.

Table 4 gives a schematic and quite approximative picture of the distribution of amino N in tissues and blood of mammals, compiled from the litterature mentioned in the introduction with the addition of the results obtained in the present paper.

My thanks are due to Professor KROGH for his constant interest in my work. I also wish to express my thanks to Mrs. KAREN SCHAUFUSS, who has assisted me with the analyses.

### Summary.

A micro method is worked out which allows the estimation of free leucine + valine in 1 g samples of tissue. The content of tyrosine and leucine + valine is determined in guinea-pig blood, liver, kidney, muscle and brain. It is found that the amino acids in question are nearly equally distributed between blood and tissues.

When leucine is injected into the blood, the concentration in the tissues is not increased over the level in the blood. Muscle and brain show a low permeability to leucine.

From the above results and from the litterature it is concluded that the high content of amino N of the tissues comes only to a minor part from amino acids. The greater part originates from glutathione and possibly other low peptides. Glutamic acid is the only amino acid which is known to be highly concentrated in the tissues as compared with the blood plasma.

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# Die Einwirkung von Alkohol auf die Atmung bei Katzen mit intaktem und denerviertem Sinus.

Von

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Eingegangen am 4 Juni 1943.

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Seit BINZ (1888) auf die stimulierende Einwirkung des Alkohols auf die Atmung hingewiesen und diesen für den therapeutischen Gebrauch empfohlen hat, sind eine grosse Anzahl experimenteller Untersuchungen ausgeführt worden, auch an Tieren, um diese Alkoholeinwirkung aufzuklären. Mehrere Verfasser meinen, dass Alkohol in kleinen Mengen keine stimulierende Wirkung auf die Atmung hat, während andere bestimmt auf eine solche bestehen. Alkohol in grösseren Mengen führt zu einer Atmungslähmung. LOEWY (1890) fand bei Versuchen mit zwei Personen, von denen die eine an Alkohol gewöhnt war, die zweite dagegen nicht, dass Alkohol bei der nicht an Alkohol gewöhnten Person die Empfindlichkeit des respiratorischen Zentrums steigerte. WENDELSTADT (1899) und FÜHNER (1932) wiesen nach, dass die Alkoholeinwirkung auf ein normales Atmungszentrum unbedeutend ist, dagegen aber bei einem kranken oder ermüdeten deutlich eine Stimulation ausübt. LEDERER und VOGT (1911) fanden in den meisten Fällen, dass bei den Versuchspersonen das Respirationsvolumen zunahm, die Atmungshäufigkeit aber unverändert blieb. WEISSENFELD (1898) zeigte, dass auf die atmungstimulierende Wirkung nach Zufuhr mässiger Alkoholmengen beim Menschen keine depressive Phase folgt. BECKMANN (1915) fand, dass nach Genuss grosser Mengen Bier eine stunden-

lang anhaltende Herabsetzung der Reizbarkeit des Atmungszentrums eintrat.

Man hat diskutiert, ob der Alkohol durch eine reflektorische Stimulation oder infolge Zunahme der Kohlensäure auf das Atmungszentrum einwirkt. JAQUET (1895) nahm an, dass die Wirkung auf einer reflektorischen Stimulation des Atmungszentrums, verursacht durch eine lokale Irritation seitens des Alkohols, speziell im Ventrikel, beruhte. WILMANNS (1897) verneinte dies und erklärte, dass die Zunahme des Atemvolumens zentral bedingt war, was er durch Injektion von Alkohol in die Arteria carotis zu zeigen vermeinte, wodurch dieselbe Zunahme erzielt wurde. SINGER (1899) war der Ansicht, dass die Atmungssteigerung nach Alkoholzufuhr auf einer Zunahme der Kohlensäureproduktion infolge des gesteigerten Stoffwechsels beruhte, und dass diese Steigerung eine Folge des durch die Vasodilatation verursachten, erhöhten Wärmeverlustes war. Dem widersprechen die Versuche WOLFERS' (1883). Er fand, dass der Stoffwechsel nach Alkoholzufuhr zunahm, selbst wenn sich das Tier in warmem Wasser befand ohne irgendwelche Steigerung der Vasodilatation. PILCHER (1911) zeigte, dass kleine Mengen 25%-igen Alkohols (2 ccm pro kg Körpergewicht) bei Katzen eine Atmungssteigerung mit 15 % hervorriefen, die 2—3 Stunden dauerte. Mit grösseren Mengen (4 ccm pro kg), die auch eine Narkose verursachten, welche zwischen tiefem Schlaf und tiefem Koma variierte, wurde die Atmungsfrequenz um etwa das Doppelte während der ersten Stunde gesteigert, ging aber stufenweise in 3—5 Stunden in die Ausgangslage zurück. Mit noch grösseren Mengen, 7.5—10 ccm pro kg, war die Frequenz gewöhnlich mehrere Stunden gesteigert, konnte aber auch unverändert bleiben. LIEB (1915) fand, dass bei decerebrierten Katzen, denen Alkohol von einer Konzentration zwischen 10—95 % in einer Menge von 0.5—5 ccm pro kg Körpergewicht zugeführt wurde, keine augenscheinliche Veränderung in der Atmung, weder an Frequenz noch Tiefe, eintrat.

HOOKER (1917) perfundierte das Rückenmark an Hunden mit defibriniertem Blut mit einer Alkoholkonzentration von 0.025 % Alkohol. Er erzielte dabei eine Steigerung der Atmung. Wurde die Alkoholkonzentration auf 0.1 % erhöht, wurde die Atmung noch mehr gesteigert. Eine Konzentration von 0.2 % verursachte eine vorübergehende Atmungssteigerung, gefolgt von einem verlängerten Atemstillstand. HIGGINS (1917) erzielte mit seinen Al-

koholversuchen an Personen bei nüchternem Magen bisweilen eine Steigerung der Empfindlichkeit des respiratorischen Zentrums, welche sich durch eine Senkung des alveolaren Kohlensäuredruckes zu erkennen gab; bisweilen war der Alkohol ohne Wirkung. Der Alkohol verringerte in den meisten Fällen das Atmungsvolumen pro Minute, was auf einer verminderten Kohlensäurereproduktion beruhte.

Gemäss den vorstehenden Verfassern ist sonach in vielen Fällen eine stimulierende Alkoholwirkung konstatiert worden. Diese Wirkung geht nach der Ansicht einiger direkt über das Respirationszentrum, nach anderen hat sie eine mehr indirekte Einwirkung auf dasselbe. In vorliegender Arbeit wurde untersucht, inwieweit die atmungsstimulierende Einwirkung des Alkohols bei Katze zu einem gewissen Teil reflexogen über den Sinus caroticus bedingt sein kann.

### Methodik.

Als Versuchstiere wurden Katzen benutzt, die als Anästhetikum 7 cg Cloralose pro kg Körpergewicht bekamen. Die Tiere wurden zuerst mit Äther betäubt und die Chloralose wurde dann in einer 1%-igen Lösung intravenös eingespritzt. Das Gewicht der Katzen variierte zwischen 2.9 und 3.2 kg.

Bei der Hälfte der Katzen wurde der Sinus caroticus vorsichtig freigelegt und denerviert, indem die vom Sinus ausgehenden Nerven durchgeschnitten wurden. Trachealkanüle und Venenkanüle in die Vena femoralis in üblicher Weise angebracht. Die Atmung wurde nach einer von EULER und LILJESTRAND (1936) angegebenen Methode registriert, in der Weise, dass das Tier in eine luftdichte Kiste gelegt wurde, die mit einem Spirometer in Verbindung stand. Die Trachealkanüle ging durch die eine Wand hinaus und stand mit einem Müllerventil mit kleinem Widerstand, einige Millimeter Wasser, in Verbindung. Die Ventilation wurde als Produkt des Spirometerausschlags und der Atmungsfrequenz erhalten. Vermehrte oder verminderte Lungenventilation gibt aber nicht immer zufriedenstellende Aufschlüsse über die Atmungstätigkeit, weshalb die Kohlensäuremenge in der Alveolarluft während der Versuche andauernd nach der von EULER und LILJESTRAND (1936) angegebenen Methode beobachtet wird. Bei den Katzen, welche Alkohol bekamen, wurde dieser in 4%-iger Lösung mit 1 ccm pro Minute in die Vena femoralis eingeführt.

## Resultate.

### I. Die Wirkung von Alkohol auf die Respiration bei Katze mit intaktem Sinus.

Wurde die Atmung bei Katzen ohne Alkoholzufuhr (3 Versuche) registriert, machte sich während einer Versuchszeit von etwa  $4\frac{1}{2}$  Stunden eine sehr deutliche, sukzessive Abnahme in der Atmungsfrequenz (von 21.5 Atemzügen in der Minute zu Beginn des Versuches bis herunter zu 10 am Ende desselben) bemerkbar, während die Amplitude beinahe konstant (14.4—14.1 mm) war. Die Ventilation nahm also, wie es sich zeigte, im Laufe des Versuches (siehe Fig. 1) sukzessiv ab, zuerst mit einem augenfällig exponentialen Verlauf, um nach etwa 1 Stunde einen geradlinigen Verlauf zu nehmen. Die Berechnung der Ventilationsgrösse auf der Kurve setzte erst nach etwa 1 Stunde ein, wenn die Ätherwirkung auf die Atmung mit Sicherheit ausgeschlossen werden konnte. An dem geradlinigen Teil der Kurve wurde die Ventilationsgrösse in 15 Perioden von  $1\frac{1}{2}$  Minute mit einem Intervall von 15 Minuten bestimmt. Das Mittel aus diesen Werten wurde als Ausdruck der durchschnittlichen Grösse der Ventilation gewählt und betrug für eine Normalkatze  $319 \pm 10.4$  ccm pro Minute.

Bei Katzen mit intravenöser Alkoholzufuhr (3 Versuche) konnte während derselben Versuchszeit nur eine unbedeutende Abnahme in der Atmungsfrequenz (18.6—14.3 Atemzüge pro Minute) und eine Amplitude (15.9—14.6 mm) beobachtet werden. Die Ventilationsgrösse betrug  $463 \pm 6.0$  ccm pro Minute, was einen Unterschied zwischen der Grösse der Atmung bei den Kontrolltieren und den Tieren mit Alkoholzufuhr von etwa 45 % (siehe Fig. 1) besagt, und dieser Unterschied, berechnet nach den üblichen Formeln der Variansanalyse (BONNIER und TEDIN, 1940) war statistisch sichergestellt ( $p = 0.001$ ). In derselben Weise wurden auch die übrigen Unterschiede berechnet.

Gleichzeitig gemachte Proben an der Alveolarluft jede halbe Stunde ergaben, dass die Kohlensäurewerte bei den Kontrolltieren zu Beginn des Versuches einen Wert von etwa 4 % haben, um gegen Ende desselben bis auf 5.2 % zu steigen. Bei den Katzen mit Alkoholzufuhr lagen die Kohlensäurewerte zwischen 4 und 4.2 %, was für eine erhöhte Reizbarkeit des Atmungszentrums bei Alkoholzufuhr spricht (siehe Fig. 2). Dies stimmt mit

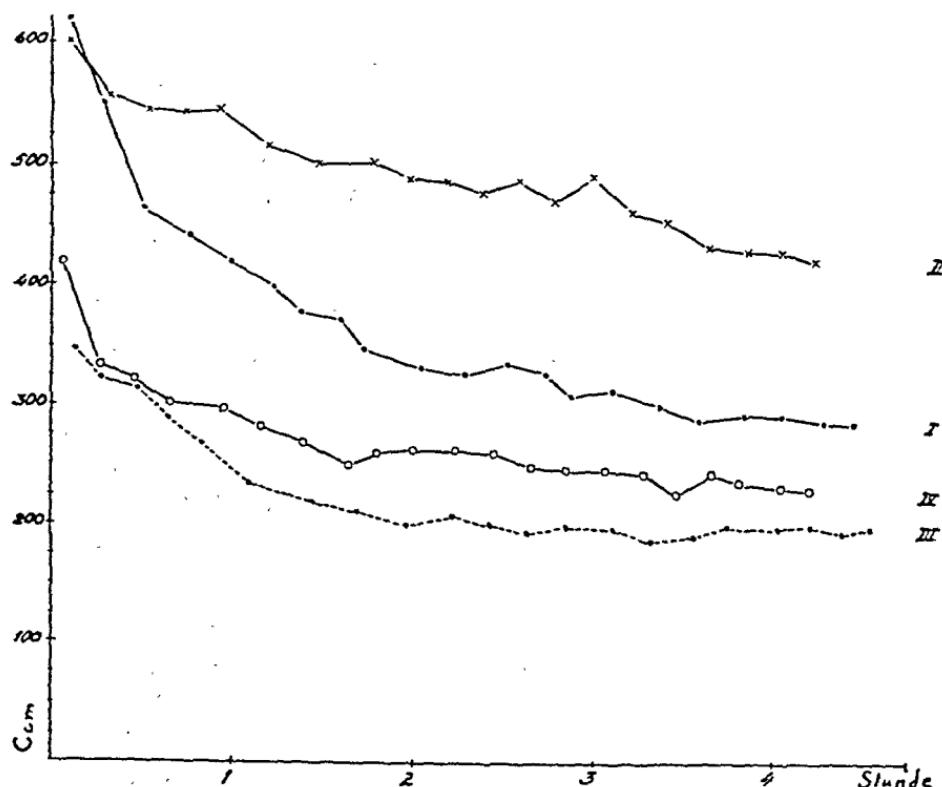


Fig. 1. I) Grösse der Ventilation bei Katze ohne Alkoholzufuhr mit intaktem Sinus caroticus. II) Intakter Sinus und Alkoholzufuhr. III) Denervierter Sinus ohne Alkoholzufuhr. IV) Denervierter Sinus mit Alkoholzufuhr.

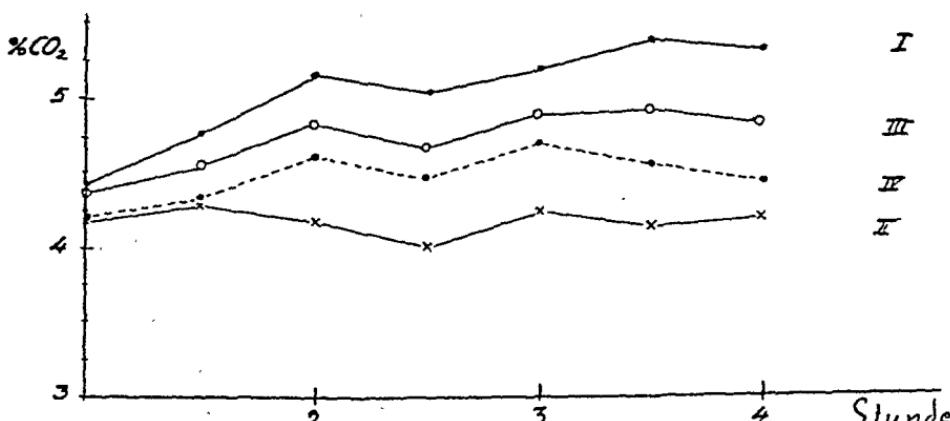


Fig. 2. I) Kohlensäuregehalt in der Alveolarluft bei Katze ohne Alkoholzufuhr mit intaktem Sinus caroticus. II) Intakter Sinus und Alkoholzufuhr. III) Denervierter Sinus ohne Alkoholzufuhr. IV) Denervierter Sinus mit Alkoholzufuhr.

den Beobachtungen mehrerer früheren Verfasser an Tieren (WOLFERS, PILCHER, HOOKER u. a.) überein.

## II. Die Wirkung von Alkohol auf die Respiration bei Katze mit denerviertem Sinus.

Bei Katze mit denerviertem Sinus sank sowohl die Atmungsfrequenz als auch die Atmungsamplitude auf ein bedeutend niedrigeres Niveau. Im Vergleich mit der Atmung bei Katze mit intaktem Sinus war der Unterschied gegen 50 %. Die Kohlensäurewerte lagen bei den denervierten Katzen höher (siehe Fig. 2).

Bei denervierten Katzen ohne Alkoholzufuhr (3 Versuche) erlitt die Frequenz der Atemzüge, wie sich herausstellte, eine sukzessive Abnahme (13.2—7.6 Atemzüge pro Minute), während sich die Amplitude nur unbedeutend (12.9—11.8 mm) verringerte.

Bei Alkoholzufuhr (3 Versuche) zeigte sich, dass die Frequenz (14—9.4 Atemzüge pro Minute) und die Amplitude (14.5—13.3 mm) nur ungedeutend geringer wurde.

Bei denervierten Katzen mit Alkoholzufuhr war die Grösse der Ventilation etwa 20 % grösser im Vergleich zu den denervierten Tieren ohne Alkoholzufuhr. Nach der Denervation hatte die stimulierende Wirkung auf die Ventilation höchst beträchtlich abgenommen, war aber immer noch statistisch wahrscheinlich ( $p = 0.05$ ).

Die Einwirkung des Alkohols auf die Atmung geht offenbar nicht nur über das Respirationszentrum, sondern ist zum Teil peripher bedingt über den Sinus caroticus.

### Zusammenfassung.

1) Es wurde die Einwirkung des Alkohols auf die Atmung an Katzen mit teils intaktem, teils denerviertem Sinus untersucht.

2) Es konnte eine stimulierende Wirkung des Alkohols auf die Atmung festgestellt werden. Die Einwirkung war am deutlichsten bei Tieren mit intaktem Sinus, aber auch bei den denervierten Tieren war eine Zunahme in der Grösse der Ventilation statistisch wahrscheinlich.

3) Die Untersuchung spricht dafür, dass die stimulierende Einwirkung des Alkohols auf die Atmung zum Teil über den Sinus caroticus geht.

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# Über eine durch Kohlenoxydbehandlung hervorgerufene qualitative Veränderung des Stoffwechsels im Froschsartorius.

Von

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Eingegangen am 8 Juni 1943.

FENN und COBB (1932 a) zeigen, dass ein Gemisch von 80 % CO + 20 % O<sub>2</sub> eine Erhöhung der Atmung von Skelett- und Herzmuskulatur und gleichzeitig damit eine Herabsetzung des Respiratorischen Quotienten bewirkt. Sie führen dies auf eine Oxydation von CO zu CO<sub>2</sub> zurück und stützen diese Auffassung durch Gasanalysen (FENN und COBB 1932 b). Dass diese Reaktion der Atmungserhöhung zu Grunde liegt, wird mit derselben Methode von STANNARD (1941) demonstriert. SEITZ (1941) führt Modellversuche aus, die evt. zur Klärung beitragen. — Ausserdem muss bei Versuchen mit CO auf Muskulatur die hemmende Wirkung auf die Cytochromoxydase in Betracht gezogen werden (STANNARD 1941, LINDAHL 1942). Die sich ergebende Grösse der Atmung beruht folglich auf dem quantitativen Verhalten der beiden Vorgänge, Erhöhung und Hemmung, zueinander.

Aus dem Obenstehenden geht hervor, dass Schlussfolgerungen auf Grund von Bestimmungen der respiratorischen Quotienten über durch CO bewirkte qualitative Veränderungen der Atmung nicht gezogen werden können. RQ-Bestimmungen können nur insofern Auskunft geben, als eine derartige Wirkung nach Entfernen des CO bestehen bleibt. Wir haben deswegen präparierte Muskeln mit einem kohlenoxydhaltigen Gasgemisch behandelt, CO sorgfältig entfernt und ihren RQ bestimmt. Da Veränderungen des RQ evt. durch Anhäufung von Milchsäure während der CO-

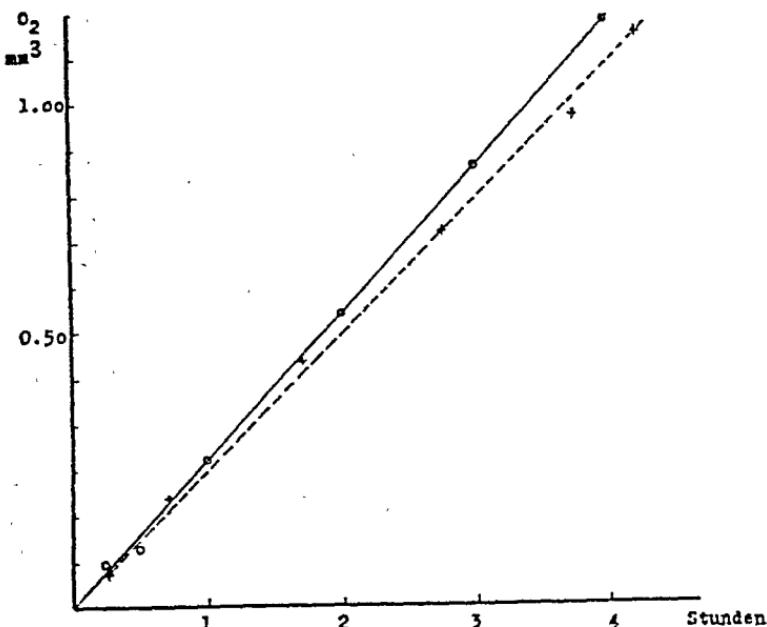


Fig. 1.  $O_2$ -verbrauch pro mg Trockengewicht in Relation zur Zeit.  
Versuch vom 10/5 1942.

○—○—○ Keine Vorbehandlung mit CO.  
+—+—+ Vorbehandlung mit CO.

Behandlung bewirkt sein könnte, wurde auch der Gehalt an Milchsäure in Kontrollmuskeln und CO-behandelten Muskeln gemessen.

### Material und Methoden.

Als Material wurden Muskeln von *Rana temporaria* verwendet und zwar M. sartorius. Die Tiere entstammten demselben Fundort und wurden teils Anfang Mai, teils Anfang September geholt. Die präparierten Muskeln wurden zunächst während 12 Stunden in eisgekühlter Ringerlösung geschüttelt. Diese Vorbehandlung ist unbedingt notwendig um die grosse individuelle Variation der Muskeln verschiedener Tiere (vgl. LINDAHL 1941) auszuschalten. Die Atmung wird nämlich hierdurch herabgesetzt, und zwar eine bei der Präparation hohe Atmung in bedeutend höherem Grade als eine niedrige. Ferner wird auf diese Weise angehäufte Milchsäure zum grossen Teil veratmet oder ausgewaschen (vgl. unten S. 244).

Der Respiratorische Quotient wurde manometrisch bestimmt. Hierfür benutzten wir eine Modifikation der Methode von MEYERHOF und SCHMITT (1929). Die Gefäße mit Gefässkonstanten für  $O_2$  zwischen 0.91 und 1.08 und für  $CO_2$  zwischen 1.11 und 1.28 sind derart konstruiert, dass Flüssigkeit aus einem Seitenanhang direkt in den Einsatz gekippt

und von hier aus in den Hauptaum entleert werden kann (vgl. WINBERG 1939). Zur CO<sub>2</sub>-Absorption wurde im Einsatz 0.3 ml einer 3 mol. NaOH-Lösung verwendet, die unter CO<sub>2</sub>-Abschluss aufbewahrt worden war. Der Anhang wurde mit 0.7 ml 20 % H<sub>2</sub>SO<sub>4</sub> beschickt, die beim Einkippen die CO<sub>2</sub> aus der Flüssigkeit in dem Einsatz, aus der Ringerlösung und den Muskeln in den Gasraum austreibt. Das Frischgewicht der Muskeln wird mit Hilfe einer Torsionswage festgestellt und für die Berechnung der Muskelvolumina — spezifisches Gewicht gleich 1.00 gesetzt — verwendet. Im Hauptaum des Warburggefäßes machen Muskel + Ringerlösung 2 ml aus. Das Trockengewicht der Muskeln wird nach 12stündiger Aufbewahrung bei 105° bestimmt.

Die Darstellung der CO geschieht nach LINDAHL (1942). Für sämtliche Versuche kam ein Gasgemisch von 80 % CO + 20 % O<sub>2</sub> zur Verwendung. Milchsäure wird kolorimetrisch nach KOENEMANN (1940) bestimmt. Unmittelbar nach Beendigung der Versuche werden die Muskeln mit Filterpapier abgewischt, schnell durchgefroren und in diesem Zustand mit einem Rasiermesser möglichst fein zerkleinert. Sonstige Vorbehandlungen, wie Bestimmung des Frischgewichtes, Fällung der Proteine und Kohlenhydrate, wird nach HANSEN, RIESER und NAGAYA (1928) vorgenommen.

### Versuche.

Die Kohlenoxydbehandlung wird folgendermassen ausgeführt. In jedes von zwei zusammengehörigen, trogförmigen Atmungsgefäßen kommen zur CO-Behandlung 2 Muskeln in 2.0 ccm Ringerlösung, und zwar ein Muskel von je 2 Versuchstieren. Zur Behandlung der Muskeln mit CO werden 500 ccm des CO—O<sub>2</sub> Gemisches während 5 Minuten beim Schütteln durch die Gefässe geleitet. Hierauf werden diese in den Thermostaten eingehängt und 60 Min. lang bei der Versuchstemperatur geschwenkt. Nach der CO-Behandlung werden die Muskeln den Gefässen entnommen, mit Ringerlösung gespült, mit Filterpapier gut abgetrocknet, gewogen und möglichst bald in die vorbereiteten Gefässe für RQ-Bestimmung überführt. Innerhalb von 15 Minuten nach der CO-Behandlung waren diese Gefässe in den Thermostaten eingehängt. Bis auf das Durchleiten des CO—O<sub>2</sub>-Gemisches werden die Kontrollmuskeln auf dieselbe Weise behandelt. Die Atmungsmessung wird 30 Min. nach dem Einhängen der Apparate in den Thermostaten angefangen. Gleichzeitig hiermit wird die Schwefelsäure in dem einen Apparat jeden Apparatenpaars eingekippt zur Messung der schon von Anfang an vorhandenen Kohlensäure. Die Messung des O<sub>2</sub>-Verbrauches dauert 4 Stunden. Nach Ablauf dieser Zeit wird die Schwefelsäure in den 2. Apparat jeden Paars eingekippt.

Der Unterschied zwischen der zuletzt erhaltenen Kohlensäure und der am Anfang der Messung erhaltenen macht die Atmungskohlensäure aus. Der  $O_2$ -Verbrauch im Laufe der Messung war konstant (vgl. Fig. 1).

Tabelle 1.

$Q_{O_2}$  und RQ von Normalmuskeln und CO-behandelten Muskeln.

Datum	$Q_{O_2}$ Normal	$Q_{O_2}$ CO-behand.	RQ Normal	RQ CO-behand.
7/3 42 . . . . .	0.27		0.79	
" " . . . . .		0.18		1.06
10/3 42 . . . . .	0.26		0.70	
" " . . . . .	0.33		0.78	
" " . . . . .		0.31		0.86
" " . . . . .		0.28		0.90
13/3 42 . . . . .	0.24		0.60	
" " . . . . .		0.17		1.07
19/3 42 . . . . .	0.19		0.68	
" " . . . . .		0.21		0.92
20/3 42 . . . . .	0.28		0.77	
" " . . . . .		0.19		0.99
21/3 42 . . . . .	0.18		0.72	
" " . . . . .		0.26		0.98
8/9 42 . . . . .	0.23		0.69	
" " . . . . .	0.26		0.73	
2/10 42 . . . . .		0.19		0.98
" " . . . . .		0.26		1.11
Mittelwert . . . . .	$0.25 \pm 0.015$	$0.23 \pm 0.017$	$0.72 \pm 0.019$	$0.99 \pm 0.028$

Tabelle 2.

Milchsäuregehalt der in O-gradiger Ringerlösung geschwenkten Muskeln vor (Kontrolle) und nach der CO-Behandlung.

Datum	Eingewogene Menge in mg	Milchsäure % Kontrolle	Milchsäure % CO-behand.	Differenz
17/9 42 . . . . .	266	0.0199		+0.0005
	279		0.0204	
17/9 42 . . . . .	203	0.0321		-0.0035
	197		0.0269	
17/9 42 . . . . .	272	0.0177		±0
	272		0.0177	
17/9 42 . . . . .	181	0.0182		±0
	183		0.0182	
1/12 42 . . . . .	278	0.0173		-0.0002
	281		0.0171	
1/12 42 . . . . .	208	0.0188		-0.0021
	198		0.0167	
1/12 42 . . . . .	179	0.0185		+0.0007
	204		0.0192	

Die Ergebnisse der Atmungsmessungen sind in Tab. 1 zusammengestellt. Unter den hier gewählten Bedingungen bewirkt die CO-Behandlung keine Veränderung der Atmungsgrösse. RQ der Kontrollmuskeln ist gleich  $0.72 \pm 0.017$ , der der CO-Behandelten Muskeln gleich  $0.99 \pm 0.028$ . Die Differenz  $0.27 \pm 0.034$  ist statistisch gesichert ( $t = 7.9$ ,  $p. < 0.001$ , vgl. BONNIER-TEDIN, 1940).

In frisch präparierten Muskeln variiert der Gehalt an Milchsäure zwischen 0.108 und 0.192 %. Wie Tab. 2 zeigt, enthalten die in der Kälte geschwenkten Muskeln viel weniger Milchsäure. Wenn die Kohlenoxydbehandlung überhaupt eine Veränderung des Milchsäuregehaltes bewirkt, dürfte es sich um eine Herabsetzung handeln ( $\frac{1}{7}$ ,  $\frac{1}{9}$  und  $\frac{1}{12}$ , Tab. 2).

### Diskussion.

In den vorliegenden Versuchen bewirkt CO keine nachträgliche Veränderung des  $O_2$ -Verbrauches. Dies steht im Gegensatz zu Versuchen, die von einem von uns ausgeführt wurden (LINDAHL 1942), für die aber frisch präparierte Muskeln verwendet wurden. Auch FENN und COBB (1932 a) geben für in Ringerlösung bei  $0^\circ$  geschwenkte Muskeln an, dass CO-Behandlung keine nachträgliche Wirkung hinterlässt. Offenbar wird irgendeine Komponente, allem Anschein nach in dem Überträgersystem — durch das langdauernde Schütteln bei  $0$  Grad verändert.

Die Kontrollmuskeln zeigen einen mittleren RQ von  $0.72 \pm 0.017$ . Der RQ des normalen Muskels ist sonst 1.0. Es liegt also auch hier eine Folge der langdauernden Vorbehandlung vor, und die Feststellung MEYERHOFS (1921), dass RQ gewaschener Muskulatur (+ Muskelkochsaft) nur etwa 0.7 beträgt, ist in diesem Zusammenhang von Interesse. Mit grosser Wahrscheinlichkeit handelt es sich in unseren Kontrollmuskeln um eine Fettoxydation. Obwohl ein Verschwinden von Fett in Froschmuskeln bei Erschöpfung normalerweise nicht stattfindet (WINFILLD 1914), kann langdauernde Reizung eine Herabsetzung des Muskel-fettes bewirken, wenn der Glykogengehalt sehr niedrig ist wie bei Sommerfröschen (NIERNIERKO 1929, BUCHWALD und CORI 1931). Die Ursache für das Ausbleiben einer Milchsäureoxydation in unseren Kontrollmuskeln trotz Anwesenheit von Milchsäure (vgl. Tab. 2) dürfte eher auf eine Veränderung des milchsäure-abbauenden Enzymsystems als auf Substratmangel zurückzu-

führen sein. Die Erhöhung des RQ durch die CO-Behandlung auf 1 kann nicht auf einer Milchsäurebildung während der Behandlung beruhen. (vgl. Tab. 2.) Entweder bewirkt die CO-Behandlung eine Restitution des Milchsäure oxydierenden Systems oder eine Mobilisierung von Glykogen, das in dem Falle nicht über Milchsäure abgebaut wird.

### Zusammenfassung.

In Sartorius-Muskeln von *Rana temporaria*, die über Nacht in Ringerlösung bei 0 Grad geschüttelt worden sind, ist RQ gleich  $0.72 \pm 0.017$ . Eine etwa einstündige Behandlung mit 20 %  $O_2 + 80\%$  CO bewirkt nach Überführung in CO-freies Medium eine Erhöhung des RQ auf  $0.99 \pm 0.028$ . Diese Veränderung kann nicht auf eine Milchsäurebildung während der Behandlung zurückgeführt werden (Tab. 2). Der der Veränderung des RQ zu Grunde liegende Mechanismus wird diskutiert.

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## Inactivation of Substance P by Tissue extracts.

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Received 11 June 1943.

In 1931 EULER and GADDUM described a substance which they extracted from the muscular coat of the intestine of the rabbit and the horse. Its chief effects were to produce a transient fall in the blood pressure and a stimulation of the isolated intestine of rabbits, even in the presence of atropine. Extracts from the brain, especially the grey matter, produced similar effects. The active principle dialyses readily through collodion or cellophane and is soluble in water, and to some extent in alcohol and acetone, but is insoluble in ether and chloroform. The chemical nature of the substance, generally known as "Substance P", was elucidated by the finding that it is precipitated by ammonium sulphate, and inactivated by trypsin, indicating that it is of a protein nature (EULER 1936). This was supported by cataphoresis experiments, which showed that substance P had amphoteric qualities with an isoelectric field within the pH range 6.5—7.0 (EULER 1942). The substance can be prepared practically free from other biologically active principles and the purest preparations so far obtained, contained one biological unit in 0.17 mg. The presence of this substance in considerable amounts in the smooth muscle tissue of the intestine and in other organs, suggest some kind of biological function. In the event of its acting as a local hormone for the regulation of the intestinal motility (EULER 1936), it seems reasonable to assume that this tissue should possess some way of breaking down the active substance.

In the present paper some experiments on the inactivation of substance P by tissue extracts of various kinds will be described.

## Methods.

In the experiments a laboratory stock preparation of substance P, obtained from horse's intestine and purified to 0.5 mg per unit, was used. It was mostly used in a concentration of 5 U. per ml in the final solution. After incubation at 38° C for various lengths of time with the tissue extract, the solutions were tested for their biological effect on the blood pressure and isolated intestine of the rabbit. The animals were anaesthetized with urethane 1.5 g/kg i. v. and had received atropine sulphate 3 mg/kg i. v. Injections were made through the external jugular vein and the pressure recorded with a Hg-manometer from the common carotid artery. The tests on the isolated rabbit's intestine were carried out on a piece of jejunum suspended in 30 ml Tyrode's solution with glucose, and aerated with 5 % CO<sub>2</sub> in oxygen. After each test the Tyrode's solution was changed and the next test was made after an interval of 5 minutes.

*Preparation of tissue extracts.* The fresh tissues were ground and extracted for a few minutes with 2 volumes of acetone. After filtration the residue was left to dry at room temperature for 24 hours. The dry substance was finely ground and extracted with 5 + 3 volumes of 2 % NaCl and centrifuged. The supernatant liquid was dialysed in running water for 24 hours and kept in the refrigerator. All mention below of the concentration of the tissue extracts refers to the amount of dry tissue used for the preparation of the extract.

## Results.

A preliminary experiment with an extract of the total small intestine of the rabbit showed a strong inactivation, which, in view of the occurrence of proteolytic enzymes in the mucous membrane, was not unexpected. Thus  $\frac{2}{3}$  of the original activity was destroyed by 15 minutes' incubation of the substance P solution with intestinal extract in a concentration corresponding to 0.03 g dry tissue pr ml at pH 6.5 and 38° C.

### 1. Extract of muscular coat of small intestine.

Further investigations were made with extracts from the muscular coat of the horse intestine. In these the mucous membrane was carefully excised from the muscular part, and the latter was freed from possible remnants of the mucous membrane by cutting off the edges.

The results of the inactivation experiments with different extracts from the muscular part of horse's intestine are collected in Table 1. In all the experiments the original concentration of

substance P was 5 U. per ml, giving a pH of 6.5 in the incubation solutions. The tests were made on the isolated jejunum of the rabbit.

Tab. 1.

	Final concentration of intestine extracts, dry tissue g/ml	Incubation time in min. at 38° C	Remaining activity (isolated intestine)
I . . . . .	0.115	10	70 %
	0.115	35	35 %
	0.115	75	10 %
II . . . . .	0.09	5	60 %
	0.09	20	50 %
	0.09	35	40 %
	0.09	50	25 %
III . . . . .	0.11	5	80 %
	0.11	20	50 %
	0.11	35	25 %
IV . . . . .	0.095	45	30 %
	0.032	20	60 %
V . . . . .	0.125	15	< 100 %
	0.125	30	50 %
	0.125	45	35 %
	0.125	65	< 25 %

Some of the extracts had faint stimulating effects of their own on the intestine, but generally this action did not disturb the tests. In doubtful cases controls were made with extract and known amounts of substance P, mixed immediately before the test.

The inactivation process was also tested on the blood pressure of the rabbit, in order to study whether the depressor effect of substance P was influenced to the same extent as the motility-promoting action on the intestine. With an extract prepared with 0.125 g dry tissue per ml (extract V in Table 1) the following results were obtained on incubation of substance P in an amount of 5 U. per ml at pH 6.5 (Table 2).

Tab. 2.

Incubation time in min. at 38° C	Remaining activity (rabbit's blood pressure)
5 . . . . .	100 %
15 . . . . .	75 %
25 . . . . .	< 50 %
40 . . . . .	< 25 %

The experiment was repeated with a solution of substance P containing 2.5 U. per ml. In this case the tests were performed with part of the extract heated to 100° C and part of it unboiled. On incubation the following results were obtained (Table 3).

Tab. 3.

Unboiled part of the extract = a Incubation time in min. at 38° C	Boiled part of the extract = b	
	Remaining activity of a	of b (rabbit's blood pressure)
15 . . . . .	100 %	1
30 . . . . .	50 %	
40 . . . . .		100 %
50 . . . . .	<20 %	
60 . . . . .		100 %

The extract itself had no action on the blood pressure with the amount used.

The rate of inactivation in the two experiments runs reasonably parallel, indicating that both actions are due to the same substance.

The effect of various pH of the incubation solution was tested in the case of the reactions of pH 4.0, 4.8, 6.0, 6.97 and 7.84. The two first pH values were obtained with buffers of citrate and HCl, the other values with buffers of primary and secondary phosphates. The following experiment with an extract of horse's intestinal muscular coat illustrates the effect.

An extract prepared with 0.094 g dry tissue per ml, was incubated at 38° C with a solution of substance P containing 5 U. per ml and buffered at the pH values mentioned above. In the final state the strength of the buffers was 0.038 M. As shown in Fig. 1, the optimal pH value of the inactivating processes lay somewhere between pH 6.0 and 6.97. Control experiments with boiled extracts were performed, and these showed that substance P itself was not interfered with by incubation at 38° at these different pH values.

## 2. Extracts from the spinal cord.

Substance P is also to be found in the spinal cord, where it is chiefly confined to the grey matter, as in the case of the brain. For that reason it seemed to be of interest to find out whether this tissue inactivates substance P.

Spinal cord of the cow was used, and extracts prepared in the

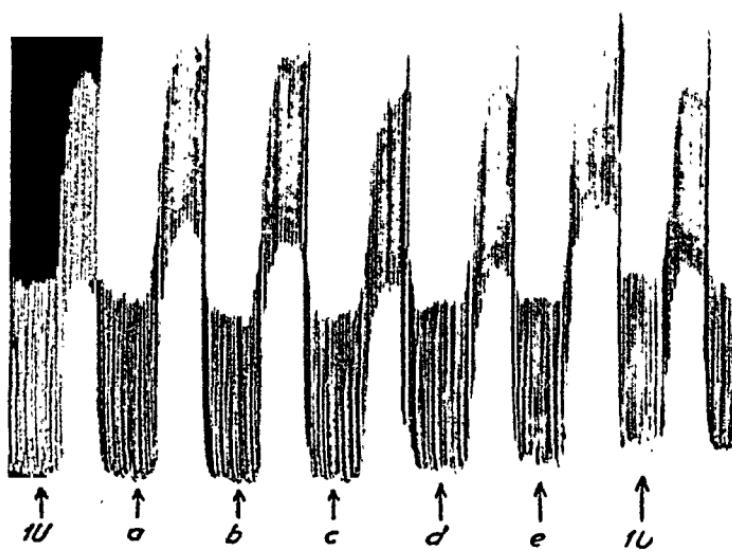


Fig. 1. Rabbit's isolated jejunum. Influence of various pH on the inactivation process with extract from horse's intestine. a, b, c, d and e correspond to pH 4.0, 4.8, 6.0, 6.97 and 7.84 respectively, and the injected incubated solutions correspond to an original amount of 2.5 U. P in 0.5 ml.

way described above. In view of the abundance of lipoid material the extraction with acetone was repeated twice. Two experiments were made.

The strength of one extract was 0.074 g per ml. On incubation with a solution of substance P containing 5 U. per ml at pH 6.5, and with the isolated jejunum of the rabbit as test object, no inactivation was observed in 55 minutes.

Another extract, the strength of which was 0.085 g dry substance per ml, on incubation at 38° C and pH 6.5 with a solution of substance P containing 5 U. per ml still showed no inactivation after 1 hour 40 min., when tested on the isolated jejunum of the rabbit. In neither experiment had the extracts any stimulating effect on the intestine. In view of the relatively small amount of grey matter and the fact that substance P appears to be present chiefly in this part, experiments were undertaken with isolated grey matter from the brain.

### *3. Extracts from the brain.*

EULER and GADDUM (1931) obtained some evidence that at least part of the activity found by JENDRASSIK (1929) and LEIM-

DÖRFER (1930) in extracts from the basal ganglia of the brain might be due to substance P. The presence of this substance in the brain has subsequently been confirmed. The following experiments were made to test whether brain tissue is able to inactivate substance P.

Basal ganglia from brain of man and horse were used, and the extracts prepared as described above.

The extracts from both kinds of brain were prepared with 0.085 g dry substance per ml. On the incubation of each extract with a solution of substance P containing 5 U. per ml at pH 6.5 the following results were obtained (Table 4). The test object was the isolated jejunum of the rabbit.

At the same time tests were made with part of the extract heated to 100° C and then incubated with a solution of substance P at the same pH and with the same amount of substance P per ml as before.

Tab. 4.

Extract	Incubation time in min. at 38° C	Remaining activity of a	activity of b
I . . . . .	15	25 %	
II . . . . .	15	25 %	100 %
I . . . . .	20		100 %
I . . . . .	30	10 %	

The extract itself from the human brain had no stimulating effect on the intestine.

The extract from the brain of horse had a slight stimulating effect on the intestine, but this effect was abolished by boiling. When judging the incubation results in this case, a comparison was made between the effect of the incubated solution which had been heated to 100° C immediately before testing, on the one hand, and the extract itself, heated to 100° C, and substance P, simultaneously but separately added to the bath and in corresponding amounts, on the other. The boiled extract did not influence the effect of substance P in this procedure, neither was the activity of substance P itself affected by boiling at this pH.

With another extract from the human brain, prepared from 0.082 g dry substance per ml, no remaining activity was found after 2 hrs 55 min. when incubated with a solution of substance

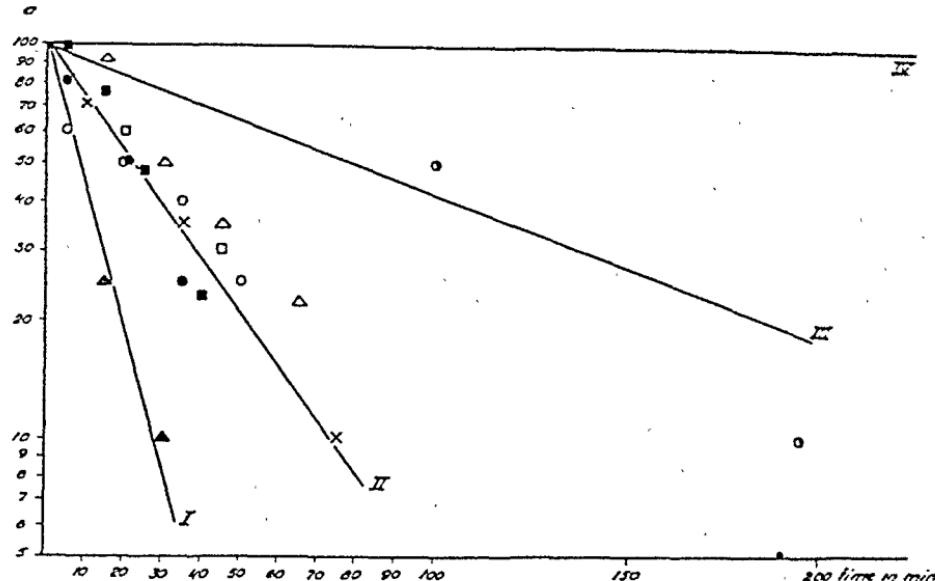


Fig. 2. The rate of inactivation with I. extract from brain, II. extract from horse's intestinal muscular coat, III. extract from striated muscle, IV. blood and extract from spinal cord.

a = remaining activity in per cent on a logarithmic scale. X extract I, ○ extract II, ● extract III, □ extract IV, Δ extract V in Table I; ■ extract in Table II; ▲ extract in Table IV; ○ extract from striated muscle.

P under the same conditions as above, whereas part of the extract heated to 100° C did not destroy any activity of substance P after 4 hours' incubation.

#### 4. Inactivation experiments with blood.

EULER and GADDUM (1931) stated that substance P was not inactivated by blood. In their experiments defibrinated goat's blood was used, and the criterion was the blood pressure of the rabbit. This question has been reinvestigated, with the use of a purified preparation of substance P.

Heparine plasma from a rabbit was incubated with a solution of substance P for various periods and was tested on the isolated small intestine of the rabbit. The plasma itself had a small but definite stimulating effect on the intestine. This effect was reduced by atropine. In order to judge of the results of the incubation, a certain amount of the incubated mixture was compared with a corresponding amount of plasma and P-standard which were simultaneously added to the bath. Even after 3½ hours at 38° C the incubated mixture still showed the same effect as the corresponding amount of plasma and P-standard directly added

to the bath. The same results were obtained with incubated solutions taken at intermediate times, showing that no inactivation took place. On one occasion it seemed as if the plasma made the intestine of the rabbit less sensitive to a following addition of P-standard to the bath. However, this lowering of the sensitivity disappeared if the usual interval of 5 minutes between the injections of plasma and P-standard was doubled.

##### 5. *Inactivation experiments with extracts from striated muscle.*

To find out whether extracts from a tissue, as distinct from blood containing only small quantities of substance P, could inactivate substance P, striated muscle was chosen.

Musculus quadriceps femoris from man was used, and the extracts prepared as usual. One experiment only was made.

The strength of the extract was 0.094 g dry substance per ml. On incubation with a solution of substance P containing 5 U. per ml at 38° C and pH 6.5, 50 % of the activity remained after 1 hour 40 min. and after 3 hrs 15 min. about 10 % of the activity still remained, when tests were made on isolated jejunum of the rabbit. The extract itself had no stimulating effect on intestine. In comparison with intestinal wall and basal ganglia, the activity of striated muscle is thus rather low.

### Discussion.

It has been shown that substance P occurs most abundantly in the muscular part of the intestine and in the grey matter of the brain. The present experiments have aimed at finding out whether these tissues are capable of inactivating substance P, as might be expected if the substance has a biological significance in these organs. The inactivation effects have been considerable; thus about half the inactivation of substance P had taken place after about 20 min. with extracts prepared from about 0.1 g dry tissue of intestinal muscular wall per ml at 38°. The inactivation appeared to an equal degree when tested on the isolated small intestine of the rabbit and in experiments on the blood-pressure of rabbits. It is interesting to compare the power of inactivation in 1 g horse's intestine with the amount of substance P which can be extracted from the corresponding amount of intestine. EULER (1942) states that in a preparation 32,000 U. P was extracted

from 10 kg horse's intestine, or 3.2 U. per g intestine. In the experiments described above about 0.1 g dry tissue, corresponding to 0.5—0.7 g fresh tissue per ml, inactivated 2.5 U. P out of 5 in about 20 min. This means that theoretically 1 g of the muscular part of the intestine can inactivate in 20 min. about  $\frac{3}{4}$  of the amount of substance P that can be extracted from it.

There seems to be little doubt that the inactivation of substance P brought about by the various tissue protein extracts is of enzymatic nature with an optimal pH between 6.0 and 6.97. This conclusion is supported partly by the methods of preparation on producing the tissue extracts, partly by control experiments with boiled tissue extracts compared with an unboiled part of the extract. We are not in a position to give much detail concerning the nature of the enzyme, but it seems highly probable that it belongs to the group of polypeptidases or cathepsines. Its occurrence in tissue extracts and its action on substance P, which is of protein nature, points in this direction. Cathepsine activity has been described in extracts of brain (Edlbacher et al.) but not, so far as we are aware, in smooth muscle tissue.

It is worthy of note that extracts from the pure muscular coat of intestine should possess such a strong inactivating effect on substance P, i. e. proteinase activity. This fact, combined with the relatively large quantities of substance P present in the muscular coat, seems to support the opinion that substance P is of physiological importance, presumably for the regulation of the motility of the intestine.

It also proved of interest to establish whether any inactivation was achieved with extracts from the central nervous system, which has proved to contain considerable quantities of substance P. No inactivation was shown with extracts from the spinal cord, but with extracts from the basal ganglia the effect was strong. Considering the fact that substance P appears most abundantly in the grey matter, comprising the chief material for the basal ganglia, it seems as if there was a certain connection between the amount of substance P and the inactivating strength of the tissue. Extracts from tissues with small inactivating power, such as striated muscle or blood, are poor in substance P.

The close correspondence in the inactivation experiments with extracts from the brain of man and horse affords reason to believe that a similar correspondence exists between extracts of other organs from man and horse.

### Summary.

The inactivating power of protein tissue extracts was studied on a purified preparation of substance P from horse's intestine.

Extracts from the muscular coat of horse's intestine, corresponding to 0.1 g dry tissue pr ml, causes half inactivation of substance P in 5 units per ml in about 20 minutes at pH 6.5 and 38° C.

Extracts from basal ganglia of brain from man and horse prepared in the same way have a still stronger inactivating power on substance P. These effects are abolished by boiling the extracts, indicating that the inactivation is of enzymatic nature. The pH-optimum of this reaction is between pH 6—7.

Extract from striated muscle proved to have comparatively feable inactivating action on substance P, whereas blood and extracts from spinal cord were without action at the concentrations studied.

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## N-methylierte Skopolaminderivate.<sup>1</sup>

Von

EBBE NYMAN

Eingegangen am 15 Juni 1943.

In einer systematischen Arbeit über den anticholinergischen Effekt einer Reihe von zur Atropingruppe gehörenden Alkaloiden hat Verf. (1942) gewisse der Beobachtungen v. ISSEKUTZ's (1917) bestätigt und erweitert, u. a. die, dass N-methyliertes Atropin (fünfwertiger Stickstoff im Tropinteil des Moleküls) in gewissen Beziehungen eine erheblich stärkere peripherer anticholinergische Wirkung hat als gewöhnliches Atropin (dreiwertiger Stickstoff). Das Methylatropinnitrat ist seit langem unter dem Patentnamen Eumydrin (BAYER) bekannt und hat als Spasmolyticum, namentlich bei der Behandlung des Pylorospasmus der Säuglinge, aber auch zu anderen Zwecken, eine ziemlich ausgedehnte Verwendung gefunden. Sonstige Methylatropine scheinen bisher in der Therapie eine sehr bescheidene Rolle gespielt zu haben.

Methylatropine mit fünfwertigem Stickstoff dürften nicht in der Natur präformiert vorkommen. Zum ersten Male wurden Verbindungen von diesem Typus Sulfat und Jodid, von BROWN und FRASER (1869) dargestellt, deren Angaben über die Eigenschaften in extenso zitiert zu werden verdienen: »We also have seen and our observations agree with those of many previous experimenters, that when a salt of atropia is administered in a large dose to a dog, the predominant symptoms are those of paralysis coexisting with convulsions. The experiments we have

<sup>1</sup> Die Untersuchung wurde mit Beitrag seitens Stiftelsen Therese und Johan Anderssons Minne ausgeführt.

now described show that convulsions are never produced by salts of methyl atropium, but that the predominating symptoms of their action are those of paralysis alone. It is therefore obvious, that by the chemical addition of jodide or sulphate of methyl, some important change has been affected in the action of atropia, by which its power to produce convulsions has been removed.

Die Darstellung von Atropinalkylnitrat wurde 1901 von BAYER patentiert (DRP. 137622 u. 138443). Im nächsten Jahr (1902) wurde das Patentrecht durch MERCK auf die Darstellung von Alkylbromiden der Tropéine und Skopoléine ausgedehnt (DRP. 145996). In der Patentschrift wird auf Untersuchungen von VAUBEL (1902) und DARIER (1902) verwiesen, laut welcher das Methylatropinbromid weniger toxisch sein soll als die gewöhnlichen Atropinsalze. Dem widerspricht eine Angabe FRÄNKELS (1901), nach der die Dosis letalis minima für Methyl- und Äthylatropin erheblich niedriger sein soll als für die gewöhnlichen Atropinsalze. Nach der letzten zur Verfügung stehenden einschlägigen Untersuchung, welche von GRAHAM und LAZARUS (1940) ausgeführt wurde, ist die Giftigkeit des Methylatropinnitrats im Tierversuch deutlich grösser als die des Atropins (bei intraperitonealer Zufuhr bei Mäusen dreimal so grosse Toxizität).

VAUBEL (1902) hat die Wirkung des Methylatropinbromids (von MERCK hergestellt) mit der des Atropinsulfats verglichen und dabei u. a. gefunden, dass die Methylverbindung als Mydriaticum sowohl beim Menschen wie der Katze einen rascher einsetzenden und wesentlich kürzeren (1/12—1/18) Effekt hat als das letztere. Ferner wurde eine geringere Beeinflussung des Herzvagus konstatiert. Auch die zentralen Wirkungen wurden als schwächer beurteilt. Über ähnliche Erfahrungen hat POHL (1904) berichtet.

Die Angaben VAUBELS stehen jedoch nicht im Einklang mit den Ergebnissen späterer Versuche mit einem anderen Methyl-derivat, dem Methylatropinnitrat (Eumydrin). Nach ERBE (1903), GOLDBERG (1903); LINDENMEYER (1903) und GRUBE (1905) ist die mydriatische Wirkung des Methylatropinnitrats zwar nicht so anhaltend wie die des Atropinsulfats, aber der Unterschied erreicht bei weitem nicht die von Vaubel angegebene Grössenordnung. Nach v. ISSEKUTZ (1917) ist Methylatropinnitrat als Mydriaticum mit Atropinsulfat in eine Reihe zu stellen, während die diesbezügliche Wirkung desselben nach

Verf. (1942) etwas stärker als die des Atropinsulfats ist (1.3 : 1). Der Effekt auf den Herzvagus stimmt auch nicht mit VAUBELS Angaben über das Bromid überein. Nach v. ISSEKUTZ (1917) führt die Umwandlung der Tropéine in quartäre Ammoniumbasen zu einer starken Steigerung ihrer Herzvaguswirkung (bei Versuchen am Frosch ca. das Achtfache). Verfs. Erfahrungen (1942) von Versuchen am Menschen lehren, dass die Herzvaguswirkung des Methylatropinnitrats wenigstens dreimal so stark ist wie die des Atropinsulfats.

Wie v. ISSEKUTZ (1917) konnte auch Verf. (1942) eine markante Steigerung der sekretionshemmenden Eigenschaften der N-methylierten Derivate im Vergleich zu den Stammverbindungen nachweisen. Die zentrale Wirkung des Methylatropinnitrats und Methylhomatropinnitrats bei Versuchen am Frosch wird von v. ISSEKUTZ mit 1/30—1/50 der betreffenden Stammverbindung angegeben.

VAUBEL (1902) schloss seine Veröffentlichung mit den Worten, er wäre mit weiteren Arbeiten über Methylatropinbronid beschäftigt und stände im Begriff, auch Äthylatropinbromid, Methylhomatropinbromid und Methylhyoscyaminbromid sowie, was in diesem Zusammenhang besonderes Interesse besitzt, Methylskopolaminbromid zu untersuchen. Die Resultate würden »demnächst zur Veröffentlichung gelangen«. Eine Fortsetzung der Vaubelschen Arbeiten war indessen im zur Verfügung stehenden Schrifttum nicht zu finden. Recht bemerkenswert erscheint daher eine Angabe in einer der obenerwähnten Patentschriften (DRP. 145996), welche aus demselben Jahr stammt wie Vaubels Arbeit (1902). In dieser heisst es: »Die Untersuchung der Bromide von ähnlichen Derivaten anderer Alkaloide der Tropéin- und Skopoléingruppe hat weiter gezeigt, dass sich diese Verbindungen auch vor den ursprünglichen Alkaloiden vorteilhaft auszeichnen, indem ihnen die unerwünschten Nebenwirkungen entweder ganz fehlen oder wenigstens stark zurücktreten, während die geschätzten Eigenschaften der Pflanzenbasen ihnen erhalten bleiben.«

Welche experimentellen Erfahrungen mit N-methylierten Skopolaminen dieser Behauptung zugrunde liegen, wird jedoch weder aus der Patentschrift noch auf dem erreichbaren Schrifttum ersichtlich. Vermutlich handelt es sich lediglich um einen experimentell nicht begründeten Analogieschluss.

FRÄNKEL (1921) hat in einer späteren Auflage seiner Alkaloid-

monographie diesen Passus in der Patentschrift wie folgt abgeändert. »Durch Einwirkung von Alkylbromid auf Atropin, Hyoscyamin, Skopolamin erhält man die entsprechenden bromwas-serstoffsäuren Salze der quartären Basen, denen die Gehirnwirkungen fehlen».

DARIER (1902) beschränkte sich darauf, die mydriatische Wirkung des Methylatropinbromids zu untersuchen, welche er in ein- bis zweiprozentigen Lösungen analog der des Atropinsulfats fand. Ein Tropfen einer 1 %igen Lösung bewirkte in Kombination mit 1 %igem Kokain eine fast maximale Mydriasis ohne nennenswerte Akkomodationslähmung.

Der Vermutung, dass die zentralen Wirkungen von sowohl Atropin- wie Skopolaminderivaten durch N-Methylierung abgeschwächt würden, muss ein grosses theoretisches und praktisches Interesse zugeschrieben werden. Hinsichtlich der N-methylierten Atropinderivate liegen so umfassende experimentelle Erfahrungen vor, von welchen im vorstehenden nur einige angeführt werden konnten, dass die Frage schon mit Sicherheit als im bejahenden Sinne beantwortet erachtet werden kann. *Die Methylatropine haben in kleineren Dosen zweifellos eine geringere Affinität zum Zentralnervensystem als die Stammverbindungen, obwohl ihre Toxizität grösser ist. Was N-methylierte Skopolaminderivate betrifft, so ist die Frage allem Anschein nach noch offen, wenn auch von verschiedener Seite unbewiesene Behauptungen über analoge Verhältnisse vorgebracht worden sind.*

Der zentrale Effekt des Atropins besteht beim Menschen zunächst in der Auslösung eines rauschähnlichen Exzitationsstadiums, eines euphorischen oder deliranten Zustands. Diese Wirkung kommt meistens jedoch erst nach Dosen zum Vorschein, welche in der Regel beträchtlich über den therapeutisch verwendeten Mengen liegen, wenn man von der Behandlung des Parkinsonismus absieht. Nach sehr grossen Dosen wird das Erregungstadium von Krämpfen und einer zunehmenden allgemeinen Lähmung des Zentralnervensystems abgelöst.

Skopolamin hingegen übt beim Menschen in sehr kleiner Dosis, ohne ein Hypnoticum im engeren Sinne zu sein, einen dämpfenden Effekt auf die Reflexerregbarkeit und Motilität aus. Diese Wirkung ist nach u. a. BURR und SNAVELY (1926) in die basalen Ganglien zu verlegen. Diese Wirkungsunterschiede zwischen Atropin und Skopolamin erschweren in gewissem Umfang einen Vergleich der durch N-Methylierung entstehenden Veränderungen

in den zentralen Eigenschaften derselben. Noch eine wichtige und augenfällige Differenz in der Wirkung des Atropins und Skopolamins muss in diesem Zusammenhang kurz gestreift werden. Während Atropin schon in ziemlich mässigen Dosen eine deutliche Herzvaguswirkung hat, scheint Skopolamin der allgemeinen Erfahrung nach in therapeutisch anwendbaren Dosen beim Menschen unter normalen Bedingungen keine sichere Beeinflussung des Herzvagus in Form von Pulzfrequenzsteigerung auszuüben, obwohl der sonstige Effekt des Mittels auf gewisse periphere cholinergische Funktionen nach Verfs. Untersuchungen (1942) dreimal so stark ist wie der des Atropinsulfats. Die Ursache dieses Unterschieds erscheint recht dunkel. Möglicherweise hängt derselbe mit der ausgesprochenen Affinität des Skopolamins zum Zentralnervensystem zusammen sowie mit der verschiedenen Verteilung des Alkaloids in den einzelnen Geweben.

Der Ausgangspunkt für Verfs. Untersuchungen über N-methylierte Skopolaminderivate bildeten also die Beobachtungen über die Steigerung der peripheren anticholinergischen Eigenschaften N-methylierter Atropinderivate sowie die gleichzeitige Abschwächung der Zentralen Wirkungen derselben, ferner die Feststellung der fehlenden Herzvaguswirkung des Skopolamins, einer Eigenschaft, deren Verfolg durch Methylierung Interesse besitzt.

### Eigene Untersuchungen.

Den vorläufigen Andeutungen, welche Verf. (1942) gemacht hatte, können jetzt systematische Untersuchungen über zwei N-methylierte Skopolamine angereiht werden, nämlich Methylskopolaminnitrat und Methylskopolaminbromid im Vergleich zu Skopolaminhydrobromid.<sup>1</sup> Dieselben sind auf Verfs. Wunsch von Pharmacia Ag., Stockholm, hergestellt worden, deren Chefehemiker, Herr Ingenieur E. Askelöf, hierbei keine Mühe gescheut hat; Verf. gestattet sich, hierfür an dieser Stelle seinen ergebensten Dank auszusprechen. Die Daten der beiden untersuchten Substanzen sind folgende:

<sup>1</sup> Im folgenden abgekürzt:

Skopolaminhydrobromid = SHB.

Methylskopolaminnitrat = MSN.

Methylskopolaminbromid = MSB.

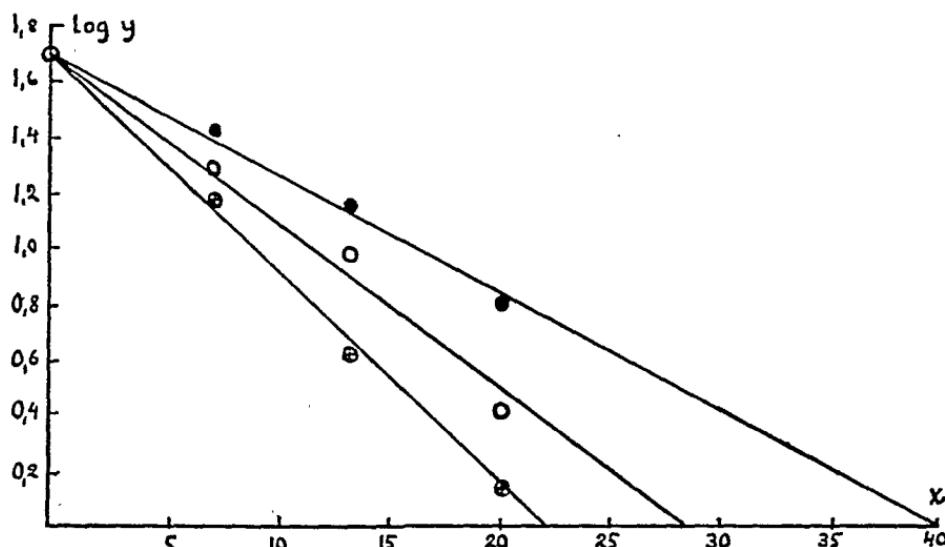
MSN.: farbloses, kristallinisches Pulver, Schmelzpunkt 179°;  
 MSB.: farbloses, kristallinisches Pulver, Schmelzpunkt 187°—189°.

Beide sind in Wasser leicht löslich. Die neuen Substanzen sind bisher folgendermassen untersucht worden:

1. antagonistische Wirkung gegen von Pilokarpin beim Menschen hervorgerufene Speichelsekretion,
2. mydriatische Wirkung beim Menschen,
3. Herzvaguswirkung beim Menschen,
4. zentrale Wirkung beim Menschen,
5. Toxizitätsprüfung an weissen Mäusen.

### *1. Hemmung der Speichelsekretion.*

Die Versuche sind an zwei gesunden Männer im Alter rund 25 Jahren ausgeführt worden. Beide hatten schon vorher als Versuchspersonen fungiert und reagierten zur Zeit der Versuche auf Pilokarpin mit konstanter Sekretion. In bezug auf Einzelheiten der Methodik sowie Grundlagen für die Berechnung der relativen



Relative Wirkungsstärke einiger Skopolaminderivate auf die von Pilokarpinhydrochlorid bei Versuchsperson E ausgelöste Speichelsekretion der einen Parotisdrüse.

y = Speichelsekretion in g während der ersten Stunde nach der Pilokarpininjektion.

x = Dosis der hemmenden Substanz in γ pro 10 kg Körpergewicht ausgedrückt.

- = Methylskopolaminbromid.
- = Skopolaminhydrobromid.
- ⊕ = Methylskopolaminnitrat.

Stärke der hemmenden Substanzen im Vergleich zum SHB. wird auf Verfs. vorangehende Arbeit (1942) verwiesen.

Die Resultate werden aus Abb. 1 ersichtlich. Ganz ähnliche Ergebnisse wurden bei der anderen Versuchsperson erhalten. Die Stärkeverhältnisse der neuen Substanzen gegenüber SHB. hinsichtlich der von Pilokarpin hervorgerufenen Speichelabsonderung aus der einen Parotis sind folgende:

Substanz	Versuchs-person E.	Versuchs-person Ö.	Mittel-wert
Methylskopolaminnitrat . . . . .	0.071	0.065	0.07
Skopolaminhydrobromid . . . . .	0.059	0.062	0.06
Methylskopolaminbromid . . . . .	0.042	0.051	0.05

Als Stärkerelationszahlen bezüglich Speichelsekretionshemmung ergeben sich also, wenn SHB. = 1 gesetzt wird, für MSN. 1.2 und für MSB. 0.8. Geht man vom Atropinsulfat (1/3 SHB.) aus, so sind die betreffenden Werte 3.6 bzw. 2.4. *Methylskopolaminnitrat ist mithin das stärkste aller bisher bekannten speichelsekretionshemmenden Pharmaka.*

In Hinblick darauf, dass MSN. die Speichelsekretion stärker hemmt als SHB., kann es verwunderlich erscheinen, dass MSB., welches doch auch fünfwertigen Stickstoff enthält, trotzdem schwächer ist als SHB. Zwecks weiterer Klärung dieses Sachverhalts wurde, teilweise in noch anderer Absicht, mit den drei Substanzen eine Vergleichende Untersuchung an 10 Männern im Alter 21—35 Jahre, welche bei verschiedenen Gelegenheiten 0.3 mg subcutan von jeder Substanz erhielten, angestellt. Die übrigen Resultate dieser vergleichenden Studie sind im Abschnitt 3 und 4 angegeben. Hier sei zur Veranschaulichung der schwächeren Wirkung des MSB. angeführt, dass sämtliche Versuchspersonen bei dieser Verbindung ein rascher vorübergehendes Trockenheitsgefühl in der Mundhöhle angaben als bei den anderen, mit Ausnahme des SHB. in ein paar Fällen. Die Dauer der Wirkung betrug laut Äusserungen der Versuchspersonen im Mittel nur ca.  $\frac{2}{3}$  von der des MSN. Dieser Umstand kann einer schnelleren Ausscheidung des MSB. zugeschrieben werden, welche ihrerseits geeignet ist, den schwächeren speichelhemmenden Effekt dieser Verbindung zu erklären.

*Mydriasis.*

Die mydriatische Wirkung der neuen Substanzen wurde in der früher von Verf. (1942) verwendeten Weise mittels des Hessischen Pupiloskops durch Bestimmung der pupillomotorischen Unterschiedsempfindlichkeit geprüft. Bei zwei Versuchspersonen war der mydriatische Effekt für beide Substanzen praktisch derselbe und gleichgross sowie ebenso anhaltend wie die Wirkung von SHB.

*3. Herzvaguswirkung.*

Bei 10 gesunden Männern im Alter 21—35 Jahre wurde der Grad der Pulsbeeinflussung nach subcutaner Injection von je 0.3 mg der neuen Substanzen sowie SHB. ermittelt. Die Pulsfrequenz wurde im Laufe von 60—80 Minuten nach der Injektion alle 10 Minuten bestimmt.

Die Resultate werden aus der folgenden Tabelle ersichtlich, in der man bei SHB. die grösste Abweichung vom Ruhepuls und bei den übrigen Verbindungen die höchste beobachtete Pulsfrequenz in Prozent des Ausgangswerts findet.

Versuchs-person	Gewicht in kg	Alter	Puls- frequenz in Ruhe	Maximale Pulsfrequenzänderung in Prozent des Ruhewerts nach 0.30 mg subcutan von:		
				Skopolamin- hydrobromid	Methylskopo- laminnitrat	Methylskopo- laminbromid
J. T. . .	76	24	62	+3	+39	+39
S. . . . .	51	25	65	-2	+54	+41
J. . . . .	65	24	60	±0	+48	+63
T. . . . .	64	21	64	+5	+35	+38
B. . . . .	75	31	60	-2	—	+53
S. S. . . .	68	25	52	+4	+73	+58
G. J. . . .	72	35	55	+7	+64	+21
H. H. . . .	70	21	66	+6	+30	+24
E. L. . . .	70	23	66	-3	+58	+30
I. S. . . .	75	24	62	—	+61	+19
Mittlere Abweichung in Prozent				+2	+51	+39

Die alte Beobachtung, dass SHB. in therapeutisch verwendbaren Dosen den Herzvagus nicht merkbar beeinflusst, wird also deutlich bestätigt, und zugleich stellt sich heraus, dass die N-methylierten Skopolaminderivate die für die ganze Atropingruppe im übrigen charakteristische Eigenschaft zurückerhalten haben, die Wirkung des Herzvagus zu hemmen. Der Effekt ist sehr markant, und wenn auch der Unterschied zwischen dem Nitrat und Bro-

mid nicht statistisch sicher ist, so ist doch die Tendenz da: das Nitrat übt auch in dieser Beziehung eine stärkere Wirkung aus als das Bromid.

#### 4. Zentraler Effekt.

Sämtliche Versuchspersonen bei den im vorigen Abschnitt (3) besprochenen Experimenten wurden auch in bezug auf die zentralen Wirkungen der einzelnen Skopolaminderivate beobachtet. Nach SHB. (0.3 mg subcutan) wiesen sie sämtlich die typische Abstumpfung und fast alle eine mehr oder weniger ausgesprochene, ataxieähnliche Gangstörung auf. *Nach den N-methylierten Derivaten war in keinem Fall auch nur eine Spur dieser Wirkungen zu entdecken.*

Um die Frage der zentralen Wirkung fernerhin zu beleuchten, wurden die einzelnen Verbindungen in Dosen von bis 0.9 mg bei einem Fall von hochgradiger arteriosklerotischer Demenz (alte Frau mit ausgesprochener motorischer Unruhe, welche monate lang unverändert bestanden hatte) subcutan injiziert. Schon nach 0.3 mg SHB. trat eine mässige Hemmung der Motorik ein, welche nach 0.6 mg ausgeprägter und von Somnolenz begleitet war; 0.9 mg versetzten die Kranke in tiefer Schlaf. Nicht einmal 0.9 mg der beiden N-methylierten Derivate konnten bei diesem Fall die motorische Unruhe auch nur andeutungsweise beeinflussen. Ebensowenig wurden andere Wirkungen als Trockenheit der Mundhöhle, Mydriasis und Tachykardie festgestellt.

#### 5. Toxizitätsbestimmung.

Diese wurde an weissen Mäusen, deren Gewicht zwischen 8 und 12 g lag, in der Weise ausgeführt, dass SHB., MSN. und MSB. pro g Körpergewicht dosiert, intraperitoneal injiziert wurden. Jede Dosis wurde an 10 Tieren geprüft. In denjenigen Fällen wo die Tiere eingingen, geschah dies regelmässig nach einem mit Atmungslähmung endenden Krampf stadium. Sämtliche Todesfälle unter den Tieren traten binnen einer Stunde nach der Injektion ein, in der Regel schon nach wenigen Minuten. Die Toxizität zeigte sich bei MSN. und MSB. ca. dreimal so gross wie die Giftigkeit des SHB., durch Vergleich der DL 50 berechnet. Das Ergebnis steht also in gutem Einklang mit der von GRAHAM und LAZARUS (1940) angegebenen Toxizitätsrelation zwischen Atropin und Methylatropinnitrat, welche 1 : 3 war.

### Zusammenfassung.

Es wird über systematische Untersuchungen zweier neuer, N-methylierter Skopolaminderivate, des Methylskopolaminitrats und Methylskopolaminbromids, berichtet. Als die Speichelsekretion hemmendes Mittel ist das erstere stärker als Skopolaminhydrobromid und damit die in dieser Beziehung stärkste bisher bekannte Verbindung. Der mydriatische Effekt entspricht im grossen ganzen dem des Skopolaminhydrobromids. Im Gegensatz zur Stammverbindung haben die beiden neuen Derivate einen markanten Einfluss auf den Herzvagus beim Menschen. Es wird gezeigt, dass diejenige Eigenschaft des Skopolaminhydrobromids, welche der sedativen Wirkung desselben auf das Zentralnervensystem des Menschen zugrunde liegt, bei den neuen Derivaten eliminiert ist. Die Toxizität für Mäuse ist auf ca. das Dreifache gesteigert.

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# The Lyophilic Properties of the Red Blood Cell Nucleus.

By

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Received 1 July 1943.

Physico-chemical and micrurgical methods have been used by various workers in attempts to obtain information concerning the colloidal system in the cell nucleus. HAMMARSTEN and TEORELL (1928) have shown that lanthanic nitrate precipitates the colloids of the red blood cell nucleus of Triton. In order to make the salt penetrate the cell membrane they added small quantities of saponin. Most of the work, however, has been performed on plant cells. WADA (1930) has observed the thixotropic properties of the nucleus and has been contradicted by PÉTERFI and KOJIMA (1936), who adopt the view that the so-called thixotropic properties are caused by the inevitable diffusion of electrolytes into the nucleus when its membrane is damaged.

In their paper PÉTERFI and KOJIMA (1936), working on the cells of *Tradescantia*, show that the nucleus is not appreciably thixotropic, that injection of water and hypertonic NaCl solution produces manifestations of hitherto unobserved structures, and finally, that injection of M/1000 acetic acid into the nucleus causes shrinkage and precipitation of the nucleus whereas M/1000 KOH applied in the same manner causes the nucleus to liquefy totally and swell considerably.

From the experiments described in this paper it will be seen that the animal cell nucleus reacts differently from that of the plant.

*Methods:* The cells were treated in a Chambers' micromanipulator with a moist chamber. The cross-sectional area of the glass tips used was not greater than  $2 \mu$ . The material consisted of red blood cells from *Rana temporaria*, whose blood cells are excellently adapted to micrurgical handling in view of their large dimensions ( $25 \mu \times 10 \mu$ ).

The blood was kept from clotting with heparin and was then suspended in various solutions and viewed in the dark field microscope. Thus observed, fresh blood cells from the frog are optically empty (cf. Figure 1). At most the outline of the nucleus is marked by a very fine bright contour. The cells were then penetrated with the glass needle. Sometimes only the outer cell membrane was pierced, in other cases the nucleus membrane also. Some observations were also performed on red blood cells suspended in solutions containing a minute quantity of saponin. In some instances after perforation the nucleus was seen to be filled with diffracting material in the form of tightly packed granules (cf. Figure 1); this was taken as evidence that precipitation of the nuclear colloids had occurred.



Fig. 1. Red blood cells from frog suspended in 0.11 M. NaCl-sol. Dark-field microscopy. Largest diameter about  $25 \mu$ . Arrow indicates cell perforated by glass needle. Note precipitated nucleus.

## Results

(+ indicates precipitation).

a) *Influence of different ions.* Approximately isotonic solutions (about 0.11 M), pH 5—8.

S o l u t i o n	Cell membrane perforated	Nucleus membrane perforated	Saponin added
Na CNS . . . . .	—	—	—
NaI . . . . .	—	—	—
NaNO <sub>3</sub> . . . . .	+	+	+
NaCl . . . . .	+	+	+
Na <sub>2</sub> SO <sub>4</sub> . . . . .	+	+	+
Na citrate . . . . .	+	+	+
Sucrose . . . . .	—	—	—
MgCl <sub>2</sub> . . . . .	+	+	+
NH <sub>4</sub> Cl . . . . .	+	+	+
NaCl . . . . .	+	+	+
KCl . . . . .	+	+	+
Frog plasma . . . . .	+	+	+
Dist. H <sub>2</sub> O . . . . .	—	—	—
FeCl <sub>3</sub> . . . . .	+	+	+
La(NO <sub>3</sub> ) <sub>3</sub> . . . . .	+	+	+

b) *Influence of concentration.*

With hypertonic (0.5 M) and hypotonic (0.05 M) solutions of the salts indicated in the first two sections of the table no precipitating effects could be seen, with the one exception that Na citrate in 0.05 M solution has a very weak effect which disappears in 0.025 M solution, as might be expected from its place in the lyotropic series (see below).

c) *Influence of pH.*

As buffer was used a 0.1 % solution of gelatine in distilled water with additions of 0.11 M acids and bases. In this buffer the salts to be investigated were dissolved in isotonic proportions.

*Na CNS* in 0.11 M solution has a pH of about 5—6 and does not precipitate the nucleus. Mixtures of NaOH and NaCNS buffer with pH 7, 8, 9, and 10 were made. At pH 9 and 10 precipitates were observed.

*Na citrate* in 0.11 M solution has a pH of about 8. Mixtures of citric acid and Na citrate buffer were made with pH 3, 4, 5, and 6. Mixtures were also made with NaOH at pH 9 and 10. In all cases precipitation occurred.

### Discussion.

It is known that the cell nucleus contains nucleohistone as predominating colloid. BANG has already shown that histone from cell nuclei is precipitated by isotonic, but not by hyper- and hypotonic solutions of NaCl and CaCl<sub>2</sub> or by distilled water. It is further known that lyophilic colloids can be precipitated by salts and that the precipitating effect of the various ions increases in the following series (at pH < 7)

Thicyanate < iodide < chlorate < nitrate < chloride < acetate  
 < sulphate < tartrate < citrate

and

Mg · · < NH<sub>4</sub> · < Na · < K · < Li ·

The effect of changing the anions is much less than is the case for the cation. In alkaline solution the series is reversed.

Considering the facts related above one is led to the conclusion that the colloids of the nucleus follow rules well known in colloid chemistry, with a few exceptions. The order of the lyotropic series cannot be reversed by shifting the pH. This may depend on the fact that the nucleus is a mixture of two colloids with optimal

precipitating tendencies at different pH, the nucleic acid at pH 3 and the histone at pH 8. Thus these critical states will dominate the picture, and precipitation will occur before the reaction has changed enough to reverse the lyotropic series. It may also be doubted whether small pH variations have any appreciable effect on the certainly strongly buffered colloidal system of the nucleus.

The precipitating effect of the plasma may be attributed to its high concentration of Cl<sup>-</sup> ions. The polyvalent heavy metal ions Fe<sup>+++</sup> and La<sup>+++</sup> have their usual precipitating effects on the proteins.

The electrically neutral sucrose does not precipitate the nucleus, nor is this to be expected.

The exact nature of the process in the nucleus, whether it be a denaturation or dehydration, a "coacervation" or something else will not be discussed here. With the facts which are at hand, the conclusions cannot be more than vague hypotheses.

The effect on the nucleus following perforation of the outer membrane occurs very quickly, and is not dependent upon whether the nucleus membrane remains untouched or is perforated by the needle. The same picture is seen when both membranes are submitted to the destructive influence of saponin. In fact, where permeability is concerned no nuclear membrane seems to exist. Several authors, however, have postulated the existence of a nuclear membrane proper. HAMBURGER and SKOWRON and SKOWRON, investigating the osmotic changes of nuclei, ascribed the volume changes observed to a membrane effect.<sup>1</sup> Volume changes, however, might equally well occur if the nucleus presented an open phase boundary to the protoplasm, the nucleus being a more compact gel suspended in the more fluid protoplasm. A piece of gelatine in salt solution may show osmotic volume changes like a more or less perfect osmometer in spite of the absence of a specific membrane.

### Summary.

1) Red blood cells from frog were micromanipulated in the presence of various electrolytes and other solutions. The induced changes were observed.

2) From the observations it was concluded that the nucleus contains a labile lyophilic system of colloids which obeys the usual

<sup>1</sup> Cited from HEILBRUNN (1936).

rules for such systems. The validity of the lyotropic series of ions was established.

3) With regard to its permeability to different ions, the nuclear membrane does not appear to exhibit any restraining or selective properties whatever.

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## The Physiological Variations in the Reaction of the Human Urine.

By

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Received 3 July 1943.

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Since the middle of last century it has been known that the reaction of the urine is changed in alkaline direction after a meal (BENCE JONES 1845). Later on it has been shown that a similar change occurs in the course of the first 3 or 4 hours after the awakening in the morning, even if no meal is consumed (LEATHES 1919, NICOLAYSEN 1932). While the occurrence of the morning alkaline tide has not been satisfactorily explained, the postprandial alkaline tide is generally supposed to be due to the secretion of acid in the stomach.

Some facts, however, seem to indicate that the postprandial alkaline tide is no simple reflection of the amount of acid secreted after a meal. ROBERTS (1859) has described the alkaline tide as beginning about one hour after the meal and lasting for a further three hours, and this has been confirmed by several other authors. Some papers even indicate that the pH of the urine may decrease during the first hour after a meal (HASSELBACH 1912, BARNETT and BLUME 1938, CAMPBELL 1920), but nobody seems to have paid much attention to this phenomenon.

Most investigations concerning the pH of the urine are carried out by means of a rather primitive technique. The purpose of the present article has been to investigate the variations in the reaction of the urine using more adequate methods and especially to ascertain, how the pH of the urine varies during the first hour after a meal.

### Experimental procedure.

pH was measured by means of a glass electrode and a Radiometer potentiometer. The urine samples were obtained by letting the person experimented on micturate into a glass bottle containing a small amount of paraffin oil. When this technique is used some carbon dioxide may be lost before the urine is covered by the paraffin oil. This will have no important influence upon the results, as shown by experiments where a part of the urine was emptied into another glass with the penis submerged in the paraffin oil. The difference in pH never exceeded 0.02—0.03 pH, even when the urine was most alkaline. As the urine samples were left in an ice-box for up to seven hours before the pH was determined, a few drops of toluene were added to prevent bacterial decomposition. No measurable change in pH was induced by storing the urine in this way.

It appears from the literature on the subject that great variations in the pH curves are found when the measurements are carried out on different persons under arbitrary conditions. In my experiments the persons experimented on lived quite regularly for periods of several days, taking their meals and sleeping at the same hours, and every unnecessary muscle activity was avoided. Thus the pH variations of the urine could be followed during several days under constant experimental conditions. It was then possible to produce average curves in which the accidental variations from day to day are to some degree eliminated, while the effect of the meals and the remaining regular variations in pH are distinctly seen. By that procedure the results were more regular and easier to explain on the individual days of the experiments too, possibly because of the fact that the meals were taken at the same hours during a long period of time.

### Experimental.

In the first experiment the pH of the urine was determined in two persons during a period of 21 days. The persons experimented on were two medical students, E. A. and C. T., both having shown a normal secretion of gastric juice. E. A. slept from 12 p.m. to 9 a.m., and C.T. from 1 a.m. to 9 a.m. To avoid interference between the alkaline tides only two meals were taken, at 12—12<sup>30</sup> p.m. and at 7—7<sup>30</sup> p.m. During the first 8 days the first meal was made rich and the second poor in proteins. During the following period of 6 days the meals were reversed, in order that the effect of one meal might be directly compared with the effect of the other taken at the same hour. During the last 7 days the food consisted of two mixed meals, and in this period a light breakfast was taken too, at 9 o'clock.

The meals were composed in the following way:

Meal I: (Rich in proteins): 370 grammes of beefsteak, three slices of bread and butter, 500 cc of milk and 200 cc of coffee.

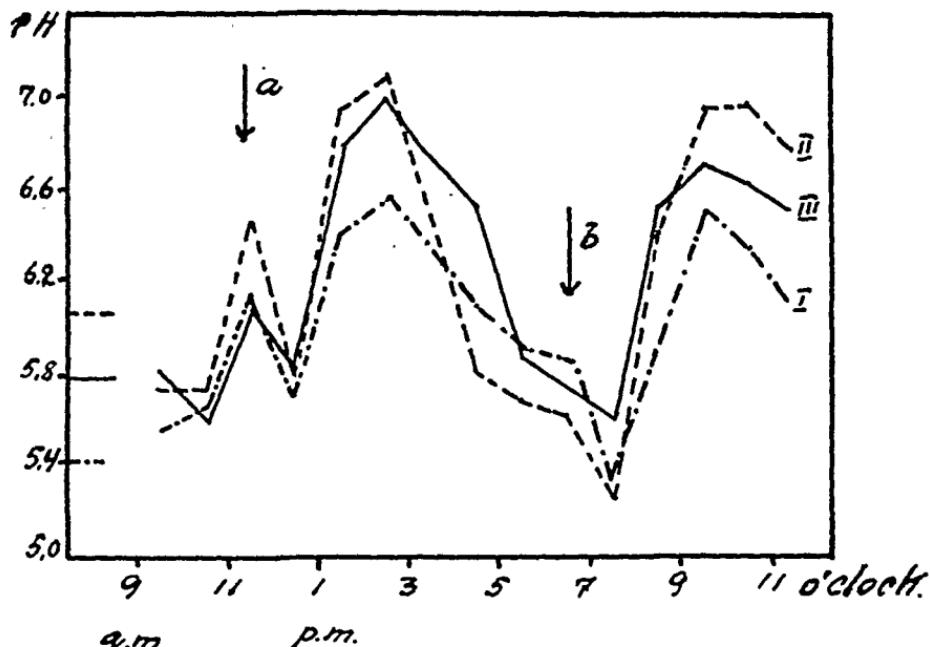


Fig. 1. The average variations in the pH of the urine during the three periods for E. A.

The abscissa gives the time in hours. The ordinate gives the pH. Curve I refers to the first period of 8 days. Curve II refers to the second period of 6 days. Curve III refers to the third period of 7 days. The horizontal lines in the left part of the figure indicate the average pH of the night urine during the three periods. a and b show the time of the meals.

Meal II: (Poor in proteins): 750 grammes of potatoes, 50 grammes of butter and 500 cc of water with a little fruit juice.

Meal III (Mixed meal): 185 grammes of beefsteak, 375 grammes of potatoes,  $1\frac{1}{2}$  slices of bread and butter, 25 grammes of butter, 250 cc of milk and 200 cc of coffee.

Breakfast: 2 slices of bread with cheese and butter and 200 cc of tea.

The results of the experiment appear from Figure 1, showing the average variations in pH during the three periods for respectively E. A. and C. T. The pH of the night urine is indicated by horizontal lines in the left part of the curves.

In spite of the fact that only three hours have passed from the awakening to the noon meal, the average curves show distinct morning alkaline tides, and these are rather more marked where no meal has been taken in the morning. In the case of C. T., the rise in pH exceeded 0.5 unit on twelve out of the 14 days, where no breakfast was taken, and in 8 cases it even exceeded 1.0 unit. With E. A. the results varied somewhat more, only 6 out of the

14 days presenting a rise exceeding 1.0 unit, and on 2 days a rise between 0.5 and 1.0 unit. During the 7 days on which food was taken in the morning a rise greater than 0.5 unit was found in 3 cases with C. T. and in 4 cases with E. A.

According to the definition of HUBBARD and MUNFORD (1925) an alkaline tide is present when the pH of at least one urine sample is more than 1.0 unit higher than the pH of one of the preceding ones, or when the pH of two consecutive samples are more than 0.5 unit higher than that of a preceding one. If this definition is applied to average curves, a marked alkaline tide is seen to follow each meal. On the individual days of the experiment the tides appeared very frequently too, being found after 31 of the 42 meals in the case of C. T., and after 38 of the meals in the case E. A. Only in four cases out of the 15 where no tide appeared the pH average was less than 6.5, in the other instances the high average may have contributed to the negative result.

A characteristic difference between the tides following Meal I and II is seen from the curves. Both in the afternoon and in the evening the alkaline tides following Meal I extend over a longer period than those following Meal II. This is particularly conspicuous in the evening, when the tide following Meal I has not ceased before bedtime. This may be the reason why the pH of the night urine is higher in the second period of the experiment than in the first. The extended rise in pH subsequent to the meal rich in proteins may be due to the fact that this meal is the more acid-binding, but on the other hand it must be emphasized that the more transient rise following Meal II may have a more vigorous character. That is the case in all instances except in the evening with C.T.

The curves of the last period during which Meal III was taken both at noon and in the evening, occupy a position between the curves of the other periods, both as regards the pH of the night urine and the height of the alkaline tides. As to the latter the evening values of C.T. once more represent an exception.

A transient fall in the pH during the first hour after the meal, before the beginning of the alkaline tide, appeared to occur frequently. The phenomenon was noted in 20 cases out of 42 in C.T. and in E. A. even in 33 cases. The average curves, too, show that phenomenon in most instances. It may be difficult to say if the decrease in pH has any direct connection with the meal, as it may be due to the fact that the alkaline tide does not begin till the second hour after the meal, while the decrease might have been

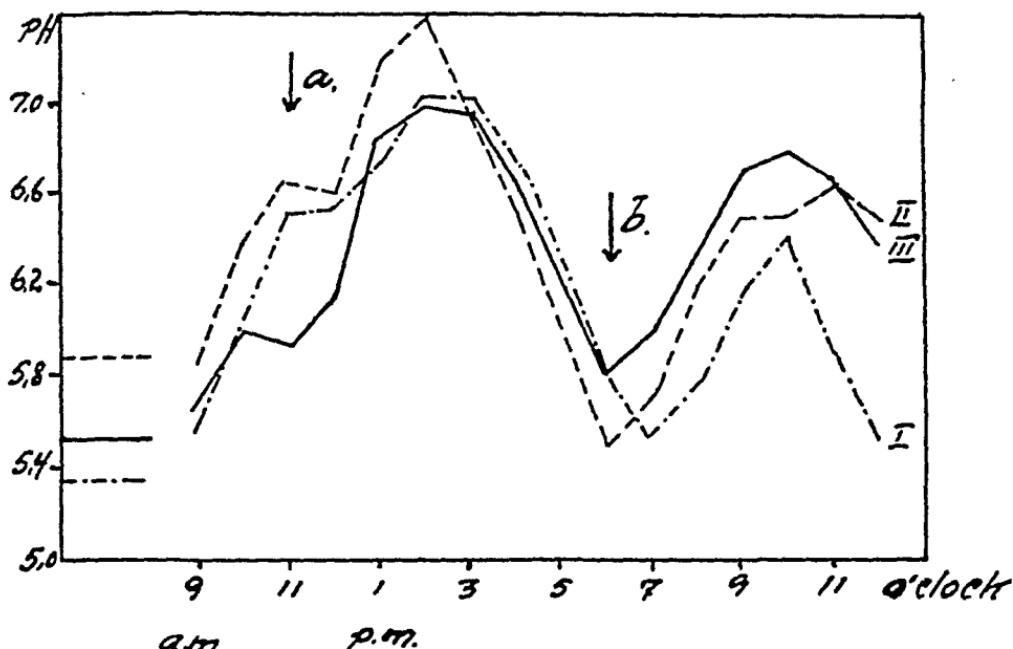


Fig. 2. The average variations in the pH of the urine during the three periods for C. T.

The abscissa gives the time in hours. The ordinate gives the pH. Curve I refers to the first period of 8 days. Curve II refers to the second period of 6 days. Curve III refers to the third period of 7 days. The horizontal lines in the left part of the figure indicate the average pH of the night urine during the three periods. a and b show the time of the meals.

found too if no meal had been taken. The evening curves of E. A., however, seem to indicate that the fall in pH is really an effect of the meal. Here the pH is decreasing before the meal, but this decrease still lessens until the time of the meal, when the curve is seen to fall abruptly. It is difficult to explain a sudden change like this in a regular curve without the assumption that the meal has been actively contributing to the acidification of the urine.

To decide definitely if a meal may produce an acid tide it was necessary to carry out an experiment where the meal was taken at a time when the pH is known to be increasing under inanition. The experiment was carried out in two divisions. In the first the morning alkaline tide was investigated during 10 days under fasting conditions. The person experimented on (E. A.) rose at 7 o'clock in the morning and did not take any food before noon. pH was measured in the night urine and in urine samples collected every hour. The average curve (Fig. 3) presents a marked morning alkaline tide, the pH increasing all the time until 12 o'clock.

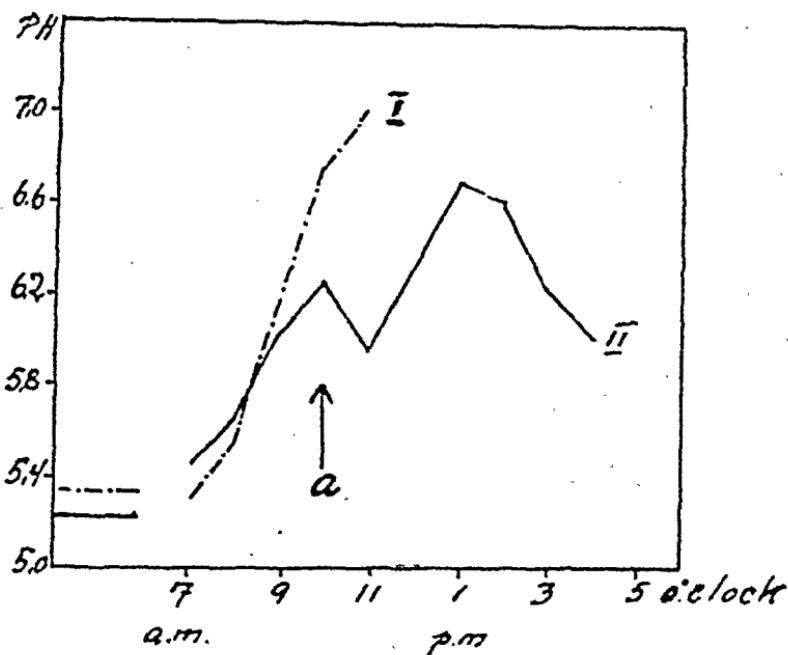


Fig. 3. The average variations in the pH of the urine under fasting conditions (Curve I) and after the taking of a meal (Curve II).

The abscissa gives the time in hours. The ordinate gives the pH. a shows the time of the meal.

During the following 5 days the experiment was continued, but now a meal was taken at 11 a. m. and the variations of the pH were followed until 5 p.m. (Fig. 3). The meal had the following composition: two boiled eggs, two slices of bread and butter, two slices of bread with butter and liver pie and 350 cc of milk. In the first division of the experiment pH had increased from 11 to 12 o'clock in 6 cases out of 10 and the average curve had shown an increase of 0.26 unit. During the last 5 days, on the other hand, pH decreased in all cases at that time of the day, with an average fall of 0.30 unit. The average curves show that the variations are approximately parallel until 11 o'clock, when one suddenly falls while the other continues its increase. After the fall in pH the usual alkaline tide ensues.

With this experiment it seems to be established that a meal may cause the transient excretion of a urine with an increased acidity.

#### Discussion.

It appears from the investigations of FISHBACK, SMITH, BERGEM, REHFUSS and HAWK (1919) that the production of hydro-

chloric acid will begin immediately on the taking of a meal and reach its maximum in the course of one hour. So the alkaline tide only makes its appearance one hour after the beginning of the secretion in the stomach and is most pronounced at a time when the alkaline secretion of the pancreas must have begun to neutralize the acid secreted by the stomach. The extensive investigations on patients suffering from achlorhydria by HUBBARD, MUNFORD and ALLEN (1924) and HUBBARD, MUNFORD (1925) seem to favour the hypothesis that the alkaline tide is due to the secretion of hydrochloric acid, which in fact must necessarily cause a more alkaline reaction in the organism, but my investigations indicate that some factor or other, present during the first hour after a meal, counteracts the increase of pH, and even in some cases brings about the secretion of a urine with an increased acidity. At present it seems impossible to ascertain the nature of this factor with any certainty, but it may be worth mentioning that HIGGINS (1914) and several other authors have stated a rise in  $pCO_2$  in the alveolar air immediately after a meal. VAN SLYKE, STILLMANN and CULLEN (1917) have stated that the quotient  $CO_2/HCO_3$  is simultaneously increasing, in other words that the reaction of the organism is getting more acid. According to APPERLY and CRABTREE (1931) and BROWNE and VINEBERG (1932) the production of hydrochloric acid depends upon the concentration of  $HCO_3$  in the blood, and every production of acid can be prevented when the contents of total  $CO_2$  in the blood is kept below a certain minimum. It may be possible, then, that the taking of a meal will reflectorily decrease the excitability of the centre of respiration in order to procure better conditions for the production of acid. If that is the case, an explanation can be given both of the increase in  $pCO_2$  of the alveolar air and of the development of an acid tide in the urine.

### Summary.

The physiological variations in the pH of the human urine have been investigated under fixed experimental conditions. The occurrence of morning alkaline tides and postprandial alkaline tides has been confirmed, and in addition it has been shown that a meal may cause a transient excretion of a urine with an increased acidity before the alkaline tide begins.

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## The Effect of Xanthine Derivatives on Parasympathetically Innervated Organs.

By

N. S. BRÄHAMMAR and N. EMMELIN.

Received 10 July 1943.

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When studying the antihistamine and antianaphylactic effect of some xanthine derivatives we observed that these substances counteract bronchoconstriction, elicited through stimulation of the vagus nerve or by means of injections of acetyl choline (EMMELIN et al. 1941). The sensitivity of the gut to acetyl choline was also diminished by these substances. The above observations may indicate that the substances in question affect the parasympathetic end mechanism. It has been established that some xanthine derivatives do affect the terminals of the autonomic nervous system (OURY 1937, BARRY 1939, BACQ and FREDERICQ 1937, 1938, 1940, FREDERICQ 1941). In order to find out how these substances worked, we investigated the effect of xanthine, caffeine and theamin (theophyllin monoethanol amine) on some other parasympathetically innervated organs.

### *The bladder.*

The experiments were performed on cats under chloralose. A cannula was inserted through the urethra into the bladder, which was then filled with Tyrode's solution at body temperature. The cannula was connected with a registering water manometer. The pelvic nerves were exposed, placed in electrodes and cut centrally. Acetyl choline was injected in the lowest part of the abdominal aorta. Histamine was given intravenously.

Fig. 1 shows one of these experiments. In all experiments the motor effect of acetyl choline, of histamine or of stimulation of the pelvic nerves was definitely lessened by administration of 25 mg. theamin per kg. body weight.

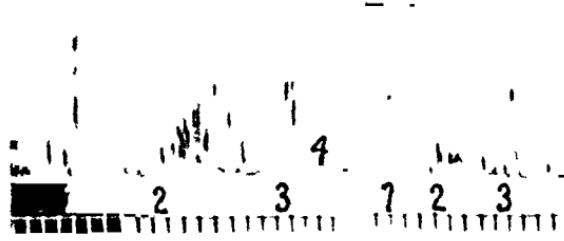


Fig. 1. Cat, 2.8 kg. The bladder motility is registered. 1 = electrical stimulation of the pelvic nerves during 5 seconds. 2 = 10 γ histamine injected intravenously. 3 = 5 γ acetyl choline intraarterially. 4 = 25 mg. theamin/kg intravenously. Time in minutes. Between the two sections of the tracing there is an interval of 5 minutes.

#### *Isolated frog's heart.*

The experiments were performed using Straub's method and cannula. The right vagus nerve was placed in an electrode. Acetyl choline and the various xanthine derivatives were added to the Ringer's solution.

There was no antagonistic effect against parasympathetical stimuli similar to that observed on smooth muscles. On the contrary, when given in high concentrations (1 : 10 000—1 : 3 000) the xanthines increased the effect of acetyl choline or of vagus stimulation on the frog's heart (fig. 2).

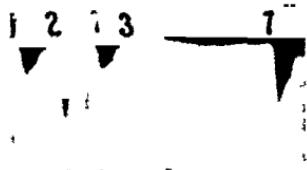


Fig. 2. Isolated frog's heart. 2 cc. Ringer's solution in the cannula. 1 = 0.005 γ acetyl choline. 2 = 0.010 γ acetyl choline. 3 = xanthine 1 : 8 000. Time in minutes.

#### *Salivary secretion.*

On cats under chloralose the submaxillary duct and the lingual nerve proximal to the chorda tympani were exposed on one side. A cannula connected with a drop recorder was inserted in the salivary duct. The nerve was placed in an electrode and cut centrally. The sympathetic nerve of the neck was also exposed and one electrode placed centrally and another peripherally to the superior cervical ganglion.

The xanthine derivatives investigated did not diminish the effect of stimulation of the chorda tympani. On the other hand fig. 3 shows that caffeine in large doses increases the salivary secretion elicited by stimulation of the chorda. This effect might be ascribed to the influence of caffeine on the sensitivity of the parasympathetical mechanism, and not to a variation of the blood flow through the gland or to a change in sensitivity within the glandular cells, as the sensitivity towards stimulation of the sympathetic nerve did not change with the administration of caffeine.



Fig. 3. Cat, 2.6 kg. The salivary secretion is registered by an electrical drop recorder. The chorda tympani is stimulated during 1 minute at 1, the sympathetic nerve in the neck praeganglionically at 2 and postganglionically at 3. 4 = intravenous injection of 75 mg caffeine/kg. Time in minutes.

### Summary.

The fact that the xanthine derivatives lessen the effect of parasympathetic stimuli on smooth muscles seems not to be due to their influence on the parasympathetic end mechanism, since a similar antagonism cannot be elicited on other structures (heart, salivary glands). As the motor effect of histamine on the bladder is diminished by xanthine derivatives one might infer that these substances act on the smooth muscle cells themselves.

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From the Laboratory for Medical Chemistry, The Medical Department, The Carolinian Hospital, Stockholm.

## An Apparatus for Obtaining an Exact Amount of Plasma from a Small Quantity of Blood.

By

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Received 3 June 1943.

In cases where a small but exact amount of blood plasma is required for analyses, the present method of obtaining the blood by veinpuncture may sometimes entail a disproportionate amount of trouble both for the patient and for the physician. Sometimes, moreover, the veins of the patient have to be saved for therapy. I have therefore constructed the apparatus described in the following, by means of which 0.1 ml of plasma may be obtained from the blood by a prick in a finger-tip or the lobe of the ear. The method can also be used for taking plasma-samples from small laboratory animals.

The apparatus (see figures) consists of a thick-walled pipette about 20 cm in length and graduated to 0.08—0.09—0.10 and 0.11 ml. Just above the graduated scale the pipette is provided with a small side-tube bent upwards (i. e. away from the tip). The whole pipette holds about 0.25 ml. The ends both of the pipette itself and of the side-tube must be ground absolutely flat. The pipettes are fitted in the arms of a special centrifuge head with an even number (2, 4 or 6) of such arms. Each arm consists of a groove into which the pipette fits. At the axial end of the groove in which the pipette is fitted is fastened a small rubber plate or cushion on which the graduated end of the pipette rests. The peripheral end of the arm can be closed by means of a small metal screw-cap, which is also provided inside with a small rubber disc or cushion. In the middle of one of the sides

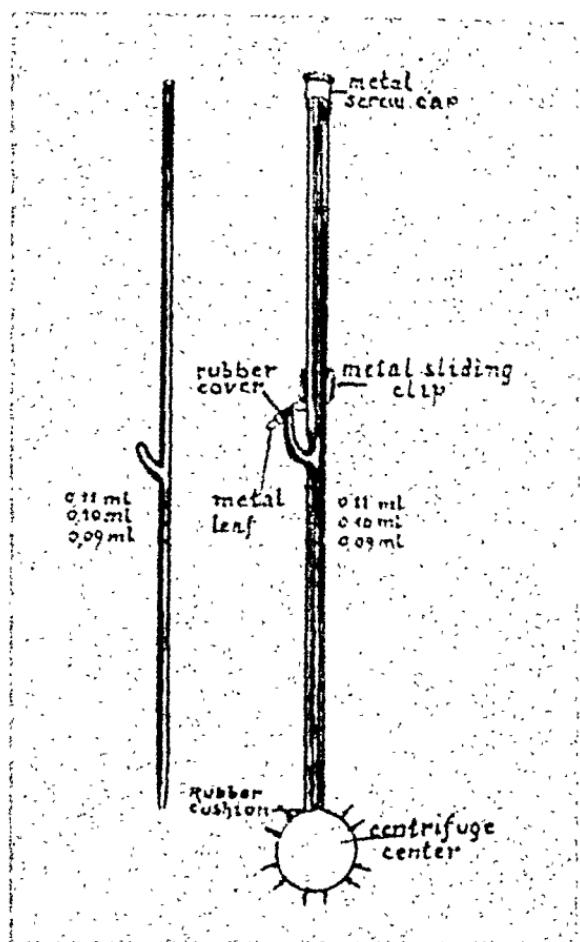


Fig. 1. The plasma-pipette by itself, and fastened in an arm of the centrifuge head.

of the groove, corresponding in position to the side-tube of the pipette, a notch is provided, about 1 cm in length. The notch is made in that side of the groove in which the side-tube rests, so that the graduation of the pipette, tip inward, becomes visible. The centrifuge arm is embraced from behind by a tightly fitting metal sliding-clip, which thus remains open over the groove. The clip is provided with a projecting metal leaf or plate. This leaf, covered with a short piece of rubber tubing, fits, when the clip is pushed towards the axis of the centrifuge head, against the end of the side-tube of the pipette. The centrifuge head can be fastened to a centrifuge making about 2,000 r. p. m.

Before use the pipette must be heparinized. The whole of the

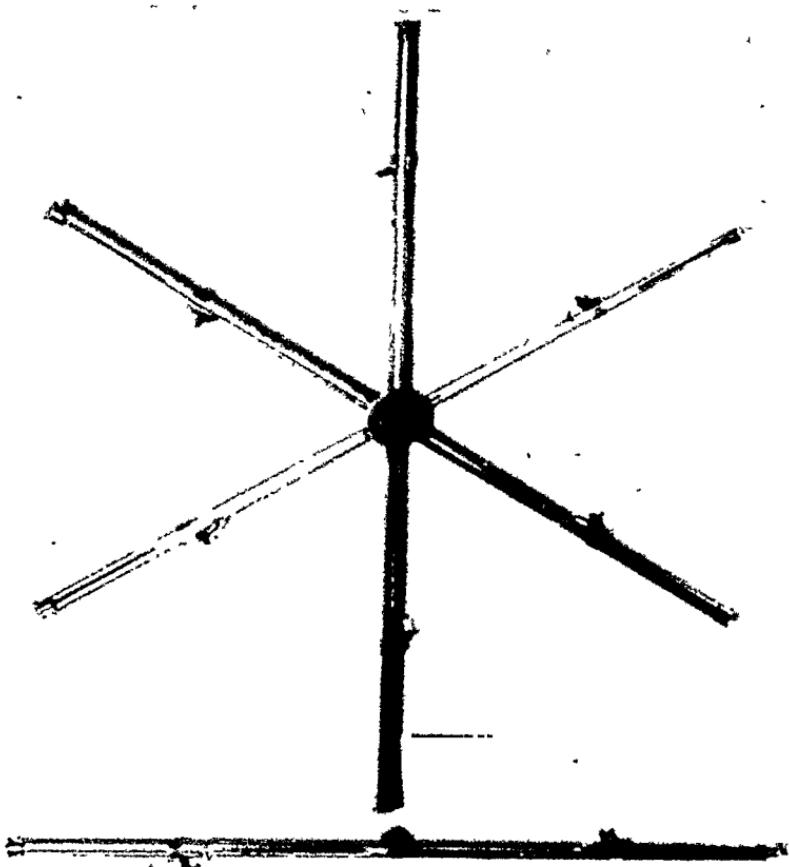


Fig. 2. Two centrifuge heads, 6 and 2 arms. One plasma pipette.

inside wall and the outside of the tip are moistened with a 5 % heparine solution and then dried at about 100° C.

When a blood column of a few centimeters is drawn into the tip of the pipette the latter usually fills itself, if held at the right angle to a copiously bleeding finger. It must be completely filled, including the side-tube. The pipette is then placed in an arm of the centrifuge head, tip inwards. While being brought into position it should be held horizontally, to prevent the blood from running out. The end-screw and the sliding-clip are pressed as tightly as possible to the upper end of the pipette and the end of the side-tube respectively. Another pipette is placed in the opposite arm, after which centrifugation is performed for about half an hour.

The centrifugation may be regarded as finished when the red cells are all on the peripheral side of the side-tube. When taking

the pipette out of the centrifuge head it should again be held in a horizontal position.

The tip of the graduated part of the pipette is now closed with one finger, while the other end is turned downwards. The erythrocyte mass runs slowly out from the open side-tube through the lower end of the pipette. The last drop is blown out with the aid of a small rubber tube attached to the side-tube. The pipette is now turned once more, the side-tube being closed by the fourth or fifth finger of the right hand, and the plasma is pipetted off as usual.

The graduation of 0.11 ml is meant to facilitate the pipetting off from a later reaction-mixture a quantity corresponding to exactly 0.10 ml of the original plasma. The 0.09 and 0.08 marks are intended as reserves in order to save the sample if the 0.10 ml mark should be passed by mistake.

The pipette can also be constructed as a hematocrit.

Two examples of the use of the pipette may be mentioned:

Total plasma protein: The nitrogen in 0.1 ml of plasma is determined by the Kjeldahl method. The result in mg of N multiplied by 6.25 gives the percentage of plasma protein.

Inulin concentration of plasma (in clearance determinations) — (modification of method described by CORCORAN and PAGE, 1940): 0.1 ml of plasma is pipetted into a centrifuge tube holding 0.8 ml  $ZnSO_4$ -solution. 0.1 ml of NaOH-solution is added. The tube is closed by rubber stopper and centrifuged hard. The clear supernatant is decanted. 0.5 ml is pipetted off into a narrow Pyrex test-tube graduated to 5 ml. 2.5 ml of diphenylamine solution is added. The tube is then heated in a boiling water-bath for 15 minutes, and the volume corrected to 5 ml by alcohol. The colour is matched in a photometer or a step-photometer.

For the composition of the reagents see CORCORAN and PAGE.

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# Eine Antriebsvorrichtung für Laboratoriumsgebrauch — in Verbindung mit einem Präzisionsinjektionsapparat.

Von

GUNNAR LINDGREN.

Eingegangen am 17. August 1943.

Bei Laboratoriumsversuchen benötigt man häufig ein Motoraggregat mit einer Welle, deren Rotationsgeschwindigkeit auch bei variierender Belastung möglichst konstant ist. Die Geschwindigkeit soll während des Versuches mit wenigen Handgriffen innerhalb weiter Grenzen zu ändern sein. An eine derartige Vorrichtung lassen sich verschiedenartige Apparaturen anschliessen (solche für Injektion, Zirkulation usw.).

Eine Kombination eines derartigen Aggregats mit einem Präzisionsinjektionsapparat ist in der Abb. 1 dargestellt.<sup>1</sup>

Ein eingekapselter Universalmotor (G) mit horizontaler Achse treibt ein System von horizontalen Zahnrädern, welche in Öl eingesenkt sind (B). Fünf der Zahnräderachsen sind länger als die übrigen (A) und oben von viereckigem Querschnitt, sodass sie ohne zu schlieren an die abnehmbare Welle (D), welche das Aggregat mit dem Injektionsapparat verbindet, gekuppelt werden können. Die Übersetzung von einer Achse auf die andere erfolgt je im Verhältnis 1 : 5. Die Geschwindigkeit des Motors lässt sich mittels des Zentrifugalregulators (L) kontinuerlich zwischen 1 und 5 einstellen. Es ergibt sich somit die Möglichkeit, die Menge pro Zeiteinheit injizierte Flüssigkeit zwischen 1 und 3 125 zu variieren, ohne die Infusion unterbrechen oder die Spritze auswechseln zu

<sup>1</sup> Die Apparatur wurde nach meinen Anweisungen von A.-B. Ardo, Stockholm, angefertigt.

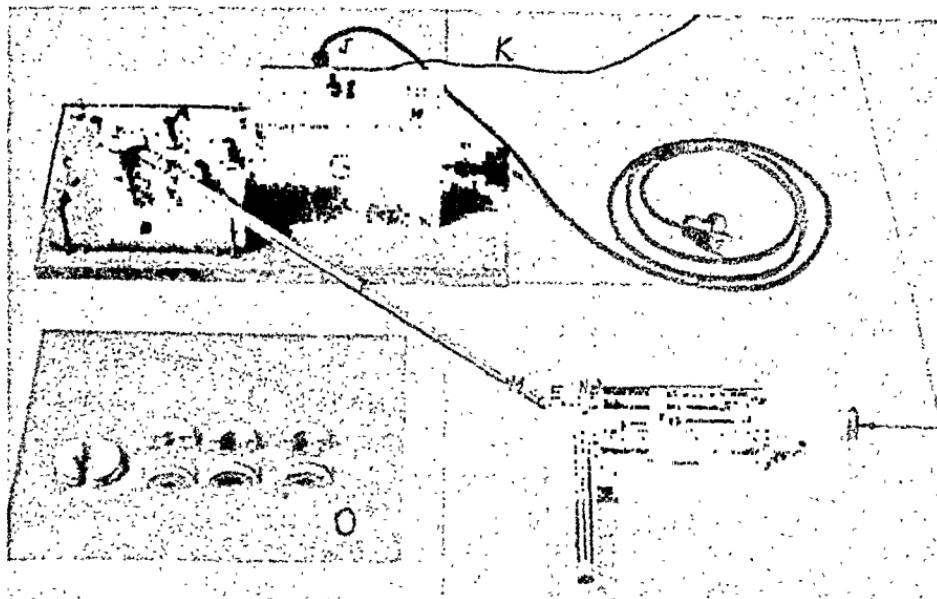


Fig. 1.

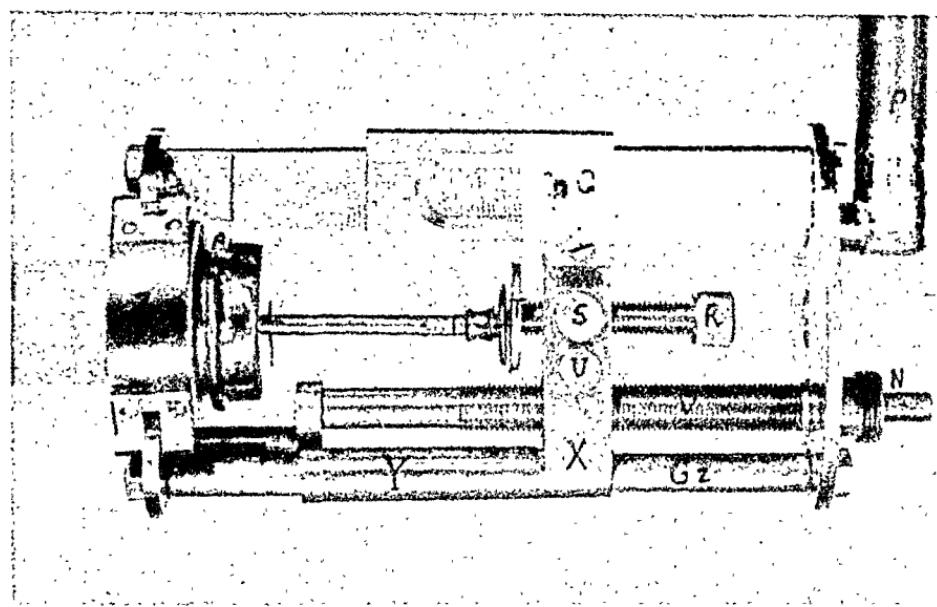


Fig. 2.

A — Zahnradachse mit oben vierseitigem Querschnitt; B — Zahnradsystem (eingekapselt); C — Gelenke; D — Welle mit eingekapseltem Zahnradsystem; E — Schalter; F — ausziehbare Achsel; G — eingekapselter Motor; H — Ventilationsöffnungen; I — Schalter; K — zur Wasserleitung; L — Zentrifugalregulator; M — Rinne; N — Verbindungsstück; O — hölzerne Einsätze; P — Griff; Q — Nonius; R — Schraube mit Platte; S — Hemmschraube; T — Platte; U — Knopf (beim Niederdrücken wird der Querbalke von der Spindel frei); V — Treibspindel; X — Querbalke; Z — Klemmvorrichtung; Gz — Führung.

müssen. Der Motor ist so kräftig, dass eine Druckzunahme von 0 auf 300 mm Hg in dem Gefäss, in welches injiziert wird, die Rotationsgeschwindigkeit nicht vermindert.

Abb. 2 zeigt den Injektionsapparat selbst. Ein durch zwei Führungen (Gz) gesteuerter Querbalke wird von einer Spindel (V) vorwärtsgetrieben. Die Bewegung ist am Nonius (Q) abzulesen. Die geringste Geschwindigkeit ist etwa 0,3 mm pro Minute. Die Spritze ist in einer besonderen Klemmvorrichtung (Z) mit auswechselbaren hölzernen Einsätzen (O) befestigt; es können daher Spritzen beliebigen Durchmessers angewandt werden. Der Kolben wird durch die Platte (T) hineingeschoben, welche sich mit der Schraube (R) in die gewünschte Stellung bringen lässt. Die Treibspindel (V) ist nicht in ihrer ganzen Länge geschnitten; bei passender Einstellung hört daher die Bewegung des Querbalkens auf, sobald die Spritze entleert ist. Beim Niederdrücken des Knopfes (U) wird der Querbalke von der Spindel frei und kann in ihre Ausgangsstellung zurückgestellt werden.

### Zusammenfassung.

Es wird eine Vorrichtung für Permanentinfusion beschrieben; dieselbe gestattet kontinuerliches Variieren der Injektionsgeschwindigkeit zwischen 1 und 3 125 — ohne Auswechseln der Spritze. Die Apparatur kann auseinandergenommen werden und der Motor mit dem Zahnradsystem für sich gebraucht werden und an verschiedene Laboratoriumsapparate gekuppelt werden.

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From the Biological Laboratories of Medicinalco Ltd,  
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## A Method for Collecting Large Samples of Blood from Living Rats.

By

CLAUS MUNK PLUM.

Received 23 June 1943.

For the investigation of the peripheral blood-picture in rats, blood is as a rule taken from a tail vein, but only small amounts can be collected in this way.

If more blood is required for experiments heart puncture has mostly been employed. This method is convenient in the case of a single experiment, but for a series of determinations on normal animals where the blood samples are taken at short intervals, or investigations on the blood of sick animals, the method is not particularly convenient since with this procedure the mortality is rather high.

As it is often of importance to be able to take 1—2 ml blood from rats at short intervals ( $\frac{1}{4}$ — $\frac{1}{2}$  hour) the following apparatus has been used for collecting the blood in this Laboratory.

The apparatus is a modification of that described by SJÖWALL (1936) for taking blood samples from rabbits' ears.

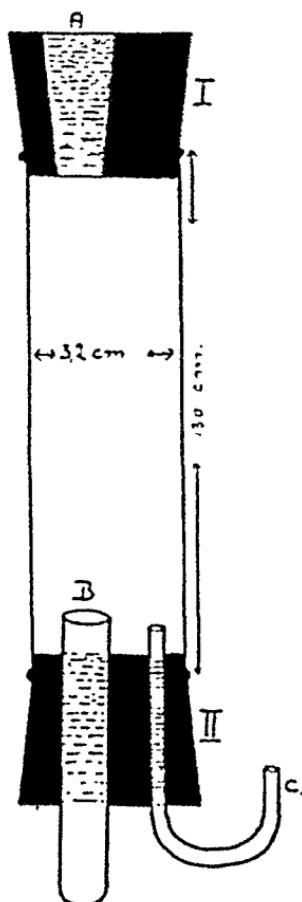
It consists of a glass tube 13 cm long, with a diameter of 3.2 cm. The glass tube is closed with two pierced rubber stoppers. In one rubber stopper (I) the hole (A) has a diameter of 1.6 cm at the free end, and a diameter of 1.0 cm at the end introduced into the tube; the other rubber stopper (II) is pierced by two holes, one with a diameter of 1.0 cm designed to hold a small test tube, the other hole being meant for a glass tube to which the vacuum pump is connected.

When the apparatus is to be used the rat is placed in such a position that its tail, well smeared with vaseline, can be passed through hole (A) (the diameter of the tail must be smaller than the diameter of hole (A) in order to avoid stasis). Immediately afterwards the glass tube is closed with rubber stopper (II) and glass tube (C) is connected to the vacuum pump, after which suction is performed for about 2 minutes. Then rubber stopper (II) is taken out and with a sharp pair of scissors  $\frac{1}{2}$  cm is cut off the tail. Now the tail is quickly put into the test tube (B), the glass tube is again closed with the rubber stopper, and suction is again put on. For practical reasons the apparatus is now held in a vertical position, and in 2—3 minutes 1—2 ml of blood will have collected in the tube (B). If the blood does not flow freely the vacuum pump can be left off at intervals.

As rats' blood clots very quickly it will be best to let a sodium citrate solution dry on the sides of the test tube and to shake the apparatus well while collecting the samples.

If blood samples are to be collected at short intervals, a little collodion cotton can be placed on the tip of the tail between the collections; but if the samples are to be taken once a day, a fresh piece of the tail must be cut off, so it will be convenient to have glass tubes of different lengths (8—13 cm).

The samples can be collected from anesthetised as well as non anesthetised animals, but a mild ether anesthesia gives the best results and does not injure the animal.



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From the Institute of Medical Chemistry, University of Upsala.

## Phosphatides in Choline Deficiency and in Adrenal Insufficiency.

By

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Received 18 July 1943.

A sure explanation of the lipotropic action of choline, i. e. of its ability to remove fat from or prevent its accumulation in the liver, has not yet been given. The probably important rôle of the phosphatides in the intermediary fat metabolism and especially in the fat transport suggests that choline acts here by entering into the phospholipid formation or, in other words, that choline deficiency limits a formation of liver phosphatides necessary for the removal of fat.

The studies of the total phosphatides in the livers of choline deficient rats fed on a high fat diet, which had already been published when the present work was undertaken, had not shown any significant quantitative changes (BEST, CHANNON and RIDOUT (1934), CHANNON, PLATT, LOACH, and SMITH (1937), LOIZIDES (1938)). BEST and his collaborators in their experiments made an additional separate determination of the choline phosphatides which were also found unchanged. The dietary periods in these experiments were relatively short (21—26 days) and the accumulation of fat only a moderate one. In the experiments of CHANNON et al. and of LOIZIDES the dietary period was also relatively short. It should be noted that in the works cited above fully grown rats were used, which seem to be much more resistant towards choline deficiency than are the younger animals.

If choline acted in the phospholipid formation, a fall in the choline phosphatides of the liver should more probably occur in long-continued periods of choline deficiency with considerable fat accumulation in the liver than in shorter experiments with

moderately fatty livers. It also appeared desirable to check with the aid of chemical analyses the determinations of choline phosphatides performed by a biological method by BEST et al. (1934). Furthermore it seemed to be of interest to extend the analyses to tissues other than the liver.

After the present work was completed I became aware of some further publications relating to the present problems, which owing to the war conditions had not been available to me before. — JACOBI, BAUMANN and MEEK (1941) found no certain change in the choline content of the liver in fully grown rats which had been maintained on a choline-deficient diet for 2—3 months. Nor was the choline content changed in the kidneys, the brain or the rest of the body. In a few young rats weighing only 35—45 g which had been maintained on a choline-free diet for only seven days, JACOBI and BAUMANN (1942) found the choline content of the whole animal at least as great as in the controls receiving choline. In the liver, indeed, it was considerably greater. On the other hand STETTEN and GRAIL (1942) also investigating young rats after choline-free dietary periods of ten days found the choline phosphatide content of the livers to be only one half that of controls given choline, while the cephalin content was unchanged or slightly increased. In the latter investigation the analyses were carried out on pooled liver samples from five animals, nothing being known about the order of the individual variations. A very interesting observation made by STETTEN and GRAIL was that the addition of sufficient quantities of methyl-consuming guanido-acetic acid in the diet brought about an almost complete disappearance of lecithin in the liver accompanied by a very marked fat accumulation. Furthermore ENGEL (1942) recently found a much lower choline phosphatide content in the livers of rats which for six weeks had received a diet deficient in choline and other lipotropic factors, than in rats which received choline in conjunction with the same diet. Thus no definite indication of the influence of choline deficiency on the quantitative relations of the liver phosphatides has been given by these latter works, which conflict somewhat in their results. The investigations made in recent years with isotopes as indicators of the metabolism of the tissue phosphatides will be discussed, as far as they are relevant, in connection with the present results.

It has been claimed that the adrenals have a choline producing (GAUTRELET (1909), LOEWI and GETTWORT (1914), HUNT (1915),

PUTSCHKOW and KIBJAKOW (1927—28) as well as a choline consuming power (VIALE (1928), and ONO (1928)). VERZÁK and LASZT (1935 and 1936) ascribe to the adrenals a phosphorylating function and have found that adrenalectomy prevents fatty livers after phosphorus poisoning or fasting. MAC KAY (1937) made the observation that after a period with a diet poor in choline the accumulated liver fat disappeared more rapidly on fasting in adrenalectomised than in normal animals. The existing indications of a rôle played by the adrenals in fat transport in the body prompted me to include some choline deficiency experiments in which adrenalectomy was performed.

### Experimental.

White rats with an initial weight of about 100 g were used, 49 in all. Of these 41 were males. Sex differences with regard to the lipids studied did not seem to occur. The rats were kept in individual glassjars at a temperature of  $+ 22^\circ \pm 1^\circ$ . The choline free basic diet used was that of GRIFFITH and WADE (1940). It is composed of 2 parts fibrin, 10 parts casein, 3 parts egg albumin, 30 parts lard (or in a few instances horse fat), 5 parts olive oil, 39 parts glucose, 4 parts OSBORNE and MENDEL's (1919) salt mixture, 2 parts agar and 5 parts cod liver oil. Other vitamin supplements were fed individually to the rats in the form of a mixture furnishing 0.02 mg thiamin, 0.02 mg lactoflavine and 0.04 mg nicotinic acid per day. About half the rats were also given 0.02—0.03 mg adermin daily.<sup>1</sup> In analyses of about 10 g of the basic diet only traces of choline could be demonstrated. According to GRIFFITH and WADE 5 mg choline per gram food was just necessary with this diet to prevent symptoms of choline deficiency.<sup>2</sup> The animals were permitted free access to the food except in the fasting experiments. All animals were given water ad libitum.

The adrenalectomies were performed according to SCHULTZER (1935) under ether narcosis. During the first day after the operation the animals were kept at about  $+ 25^\circ$ . The condition of the rats was good until the 4—7th day after the operation, when they began to show obvious signs of adrenal deficiency.

All the rats were killed with a blow in the neck, as a rule 20 hours after the last feeding. The tissue samples analysed were frozen in the fresh state and cut with a microtome into slices of about  $15\ \mu$  thickness. The details of analytical procedure will be published elsewhere. In the lipid extract (alcohol-ether 3: 1) the phosphorus was determined according to TEORELL (1931). Choline was, after alkaline hydrolysis, determined colorimetrically as reineckate according to a procedure modified after BEATTIE (1936) by the author (BRANTE 1940). Dry fat-

<sup>1</sup> For the vitamin-preparations I am indebted to A. B. Pharmacia, Stockholm.

<sup>2</sup> It should be noted that because of its protein content this diet is only moderately deficient in labile methyl-groups.

free substance was determined by weighing. The total phosphatides were calculated from lipid-P by multiplication by 25, the choline phosphatides were calculated from the lipid choline and the cephalins by difference.

As the individual variations may be rather great each animal was analysed separately. In the case of the livers all analyses were performed in duplicate on two different samples.

As the accumulation of fat in the liver appears to increase the liver's weight by about the amount of fat deposited there, the analytical values are best calculated on the lipid-free dry substance, or, as the body weight does not seem to be influenced by variations in the choline supply, with respect to some unit of body weight. As the first mode of calculation includes the analytical errors in the determinations of total fat and dry substance respectively, the latter mode was preferred. It proved to give lower divergencies in the analytical values.

The rats in the main choline deficiency group were kept on the choline-free basic diet for 104—177 days (in one rat for 85 days only), thus for a considerable time. The rats of the main control group were given 5 mg choline per gram food. All animals were in good condition throughout the experiments, the choline deficiency rats increasing in weight at the same rate as the controls.

In addition to the chemical analyses, determinations were made on the osmotic resistance of the red blood corpuscles. Such determinations were thought capable of indicating a possible influence of choline deficiency on the phosphatide distribution of the corpuscles. The technique was the following. The blood was taken by means of heart puncture and heparinised. After centrifugation and careful removal by suction of the plasma, 0.02 ml of the corpuscles were pipetted into micro test tubes and mixed with 1 ml salt solution (NaCl and KCl resp.) of various strengths. The readings of maximum, minimum and average resistance of the corpuscles were made after keeping the mixtures for 4 hrs at 18—20°.

All the analyses were performed by the author personally.

For his kind assistance in the adrenalectomies I wish to thank med. cand. P. A. Westling.

### Results.

The results obtained on liver and kidneys in the main experiment are given in Table 1.

The average values for total liver phosphatides is somewhat higher in the choline-deficient rats than in the controls. The difference  $0.29 \pm 0.11$  is, however, not quite significant.<sup>1</sup> The

<sup>1</sup> The usual equations for the calculation of standard deviation and standard error of the mean require a somewhat greater number of observations for their application than those in my experimental series of 8—10 analyses. The standard errors of the mean given in the table and the standard errors of the differences in the text have been calculated in the usual way (DAHLBERG 1910) and are therefore somewhat smaller than the true values.

Table 1.  
All values except those for total fat are in mg per g of body weight.

Choline deficient rats						Control rats, fed 5 mg choline per gm basic diet						
Rat No.	Body weight gm	"Neutral fat" mg per gm body weight	Total fat %	Choline phosphatides	Cepha-	Rat No.	Body weight gm	"Neutral fat" mg per gm body weight	Total fat %	Choline phosphatides	Cepha-	Choline Phos-
				phosphatides/total phosphatides	lins			phosphatides/total phosphatides		lins		phosphatides/total phosphatides
LIVER												
1	150	6.92	20.2	0.45	0.96	1	146	1.7	8.3	0.72	0.66	52.2
2	151	17.1	27.0	0.91	1.22	2	153	1.5	7.4	0.70	0.62	63.3
3	180	11.5	22.1	0.59	1.19	3	169	1.4	7.3	0.50	0.59	49.8
4	188	10.2	24.0	0.63	0.88	4	170	0.8	6.8	0.72	0.71	49.4
5	193	13.4	29.8	0.58	0.93	5	179	1.2	7.0	0.69	0.94	42.3
6	208	9.0	24.2	0.53	0.76	6 <sup>a</sup>	185	2.0	9.1	0.79	0.82	49.1
7	233	15.0	26.9	0.63	1.20	7	190	1.9	8.2	0.58	0.65	47.0
8	234	10.0	24.4	0.61	0.91	8	209	1.3	6.6	0.60	0.57	51.1
						9	216	1.6	8.1	0.61	0.73	45.5
						10	234	1.0	9.9	0.50	0.52	48.9
Total phosphatides						Total phosphatides						
Mean values	1.624	± 0.094	0.615	1.009	38.9			1.332	0.050	0.682	48.9	
			± 0.050	± 0.090	± 1.13			± 0.06	± 0.028	± 0.010	± 1.04	
KIDNEYS												
1	0.22	6.7	—	—	—	1	0.32	6.6	0.13	0.12	53.2	
2	0.39	7.5	—	—	—	2	0.22	6.3	—	—	—	
3	0.17	5.5	0.11	0.19	37.4	3	0.35	6.6	0.12	0.16	43.2	
4	0.16	5.7	0.10	0.17	37.0	4	0.20	6.2	0.12	0.16	40.7	
5	0.39	9.2	0.11	0.20	35.1	5	—	—	0.13	0.19	37.7	
6	0.23	6.3	0.12	0.13	47.3	6 <sup>a</sup>	0.29	6.5	0.13	0.17	42.0	
7	0.27	6.3	0.11	0.18	37.5	6b	168	—	0.13	0.12	51.2	
8	0.37	7.3	0.12	0.12	50.8	7	0.21	6.4	0.16	0.13	42.6	
						8	0.27	6.6	0.10	0.13	43.5	
						9	0.20	6.3	0.13	0.17	44.0	
						10	0.37	8.1	0.12	0.17	41.0	
Total phosphatides						Total phosphatides						
Mean values	0.270	0.110	0.166	40.9	—			0.277	0.122	0.151	43.0	
	± 0.010	—	—	—	—			± 0.008	± 0.003	± 0.008	± 1.5	

<sup>a</sup> Hero and in others tables and in the text "neutral fat" signifies: total fat — phospholipids.

mean value for the choline phosphatides is practically the same in both groups. The cephalin average is clearly higher in the choline-deficient group, the difference  $0.33 \pm 0.07$  no doubt being significant. The ratio choline phosphatides/total phosphatides is also clearly different in the two groups, the difference  $10.0 \pm 1.5$  certainly being real. There is, as can be seen, a considerable accumulation of fat in the livers of the choline deficient animals. As to the kidneys, no differences can be established between the control group and the group with choline-free diet. This last result as well as that for the liver choline phosphatides thus agree with the findings of JACOBI et al. which were cited in the introduction.

The analyses of the small intestine, the spleen and the muscles are too few to permit any definite conclusions. Table 2. Marked changes as a consequence of the choline deficiency can, however, be excluded. The low values for the choline phosphatides of the intestine in the choline-deficient rats in comparison with the controls can, however, hardly be due to chance. The analytical results in addition show that the ratio choline phosphatides/total

Table 2.

Choline-deficient rats				Control rats, fed 5 mg choline per gram basal diet			
Rat No.	Choline phosphatides %	Cephalins %	Choline phosphatides/total phosphatides	Rat No.	Choline phosphatides %	Cephalins %	Choline phosphatides/total phosphatides
<b>M U S C L E</b>							
1	0.61	0.81	42.8	1	0.67	0.89	42.6
2	0.82	0.69	54.1	3	0.75	0.76	39.7
3	0.66	0.75	46.6	4	0.60	0.75	44.2
6	0.66	0.68	49.1	6a	0.59	0.78	43.4
7	0.55	0.94	37.9	8	0.77	0.80	49.1
				9	0.62	0.79	43.6
Mean	0.66	0.77			0.67	0.79	
<b>S P L E E N</b>							
3	1.69	1.43	43.0	5	0.77	2.22	25.8
				6a	0.90	1.34	40.2
<b>S M A L L I N T E S T I N E</b>							
3	3.41	4.59	43.7	1	4.97	—	—
5	3.59	4.91	42.3	5	4.19	6.26	40.1
				6b	4.19	3.86	53.8
				10	4.23	5.02	45.8

\* The values for the small intestine are calculated in percent of dry tissue, the others in percent of fresh tissue.

phosphatides is normally about the same in the liver, intestine and muscles and somewhat lower in the kidney and possibly in the spleen.

It appears that in the individual animals the ratios between choline phosphatides and total phosphatides (or choline phosphatides and cephalins) agree in some degree for the different tissues. A high value of this ratio in one tissue is thus often accompanied by relatively high values of the same ratio in the other tissues.

Some animals were analysed under somewhat different experimental conditions than those employed in the main groups. Thus two animals were given 15 mg choline pr gram of the basic diet. The livers of these rats contained still less "neutral fat" (0.34 and 0.57 mg per gm of body weight) than the rats which had been given 5 mg choline per gram food, but otherwise displayed no obvious differences from the latter.

Four animals were maintained on the choline free diet for only 14—68 days. While the "neutral fat" during that time had increased to 2.9—4.5 mg per gm body weight (= 10—15 % of fresh tissue) the values for the liver choline phosphatides and cephalins agreed much more with the mean values of the control group than with those of the long-continued choline deficiency group. This in good agreement with the results of BEST et al. (1934), mentioned in the introduction, where the fat accumulation was of the same order. The choline phospholipid content in the small intestine was in all these four rats lower than 4 % of the weight of dry tissue, with an average of 3.73 % i. e. definitely lower than in the control rats.

Two animals which had been fed with 5 mg choline per gm food and two animals fed on a choline free diet for 130 and 174 days respectively were starved for the last 10—12 days of the experiments. The analyses of their livers are given in Table 3 together with the results obtained for the adrenalectomised animals. The fasting animals, also served as controls for the adrenalectomised ones, as these in consequence of the anorexia became markedly emaciated before death. In all fasting animals the choline phosphatides as well as the cephalins in the liver were markedly reduced and in the same degree, the ratio between them remaining unchanged (which agrees with the findings of MAC LACHLAN, HODGE, BLOOR, WELCH, TRUAX and TAYLOR 1942). In the choline-fed fasting animals the "neutral fat" in the liver was not lowered materially. In the fasting choline-deficient animals, on the other

Table 3.

*Analyses of the livers of fasting and adrenalectomised rats  
(calculated in mg/gm body weight).*

	Body weight	% loss of weight after operation or during fasting	Total fat %	"Neutral fat" mg/gm body weight	Choline phosphatides	Copha-lins	Choline phosphatides total phosphatides %
<i>Starved rats</i>							
Choline-deficient rats	138	23	16.8	5.17	0.47	0.62	43.1
	163	19	12.8	4.52	0.46	0.75	38.3
Rats fed 5 mg choline per gm food	95	36	6.6	1.19	0.42	0.46	47.1
	136	25	7.1	1.18	0.51	0.59	46.5
<i>Rats operated on but not adrenalectomised</i>							
Choline-deficient rat	199	0.5	16.0	4.82	0.65	0.71	48.1
Rat fed 5 mg choline per gm food	151	20	7.6	1.16	0.57	0.68	42.7
<i>Adrenalectomised rats</i>							
Choline-deficient rats <sup>a</sup>	175	8	9.0	1.93	0.67	0.73	47.8
	180	12	13.7	4.40	0.65	0.61	51.5
	110 <sup>b</sup>	20	6.3	1.25	0.44	0.39	53.2
	107 <sup>b</sup>	25	6.5	1.01	0.55	0.65	41.4
	125 <sup>b</sup>	27	5.4	0.72	0.50	0.76	37.5
	95 <sup>b</sup>	26	4.8	0.71	—	0.92	—
	157	22	7.2	1.25	0.36	0.62	35.8
Rats fed 5 mg choline per gm food	108	0	—	—	1.09	0.69	61
	127	9	—	—	0.66	—	—
	128	11	5.8	0.92	0.51	0.69	42.7
	202	11	7.7	1.25	0.59	0.51	52.1
	216	11	6.7	0.94	0.62	0.53	53.0
	111	15	—	—	0.71	—	—
	108	22	—	—	0.68	0.62	52.3
	113	18	7.6	1.38	0.47	0.60	43.9
	114	22	6.5	1.95	0.55	0.51	50.7

hand, the fasting brought about a marked reduction of the "neutral fat" content. In the kidneys, the small intestine and the muscles fasting did not bring about any lipid changes.

The adrenalectomised animals did not exhibit any marked changes in the lipid constituents of the liver, when compared with the fasting controls or with rats operated on similarly but without removing the adrenals. In one point only was there probably a difference. In the adrenalectomised choline-deficient rats the "neutral fat" of the liver seemed to disappear more rapidly

<sup>a</sup> These rats had been maintained on choline-free diet for only a relatively short time (2 to 6.2 days).

than in the controls. This result thus agrees with MAC KAY's. Furthermore it is worth considering that in the choline deficient rats which were operated on with or without adrenalectomy, there was an early and rather large fall in the liver cephalins. This might have been an effect of the narcosis or the traumatisation during the operation.

The determinations of the osmotic resistance of the red blood corpuscles are given in Table 4. It clearly appears that there is an increased resistance in the choline-deficient group as compared with the control group. Fasting appears to lower the resistance in the control group but not in the choline-deficient group.

Table 4.

*The average osmotic resistance of the red blood corpuscles of choline-deficient and control rats.*

	No.	NaCl	Mean	KCl	Mean
Choline deficient rats . . .	1	0.40		0.54	
	2	0.39	0.410	0.53	0.554
	6	0.43	(0.39—0.43)	0.57	(0.54—0.57)
	7	0.41		0.57	
	8	0.42		0.56	
± fasting . . . . .	9	0.40	0.410	0.51	0.545
	10	0.42		0.55	
Control rats fed 5 mg choline per gm basic diet	2	0.45		0.62	
	3	0.45		0.60	
	4	0.44		0.59	
	6a	0.43	0.449	0.58	0.600
	7	0.41	(0.43—0.47)	0.59	(0.58—0.64)
	8	0.47		0.64	
	10	0.43		0.62	
	11	0.45		0.58	
	12	0.46		0.58	
± fasting . . . . .	13	0.50	0.505	0.63	0.645
	14	0.51		0.66	

### Discussion.

The results obtained do not positively support the view that the lipotropic action of choline is due to phospholipid formation. A diet practically free from choline (but containing in the protein certain other methyl donors), maintained for several months and producing fatty livers with 25 % total fat did not bring about

any decrease in the choline phosphatides of the liver. This result does not, of course, necessarily contradict the view mentioned above. Investigations with the aid of isotopes have especially shown that the metabolism of the liver phospholipids is relatively rapid (PERLMAN, RUBEN, CHAIKOFF 1937—38) and that it is accelerated by choline (PERLMAN and CHAIKOFF 1939 a). It might well be conceived that the *rate of the metabolism* of the choline phosphatides of the liver is generally the dominating factor in the transfer of neutral fat by the liver rather than the *amount or level* of choline phosphatides in the liver cells, (compare PERLMAN and CHAIKOFF 1939 b). In choline deficiency the rate of metabolism of the choline phosphatides may possibly be lowered greatly before the amount of these substances in the liver begins to fall. Such a fall seems to take place in choline deficiency only when the supply or availability of labile methyl is very much reduced, as in the experiments of STETTEN and GRAIL (1942) with guanido-acetic acid.

Opposed to the view that choline in the diet influences the rate of choline phosphatide formation in the liver is the observation by STETTEN (1942) that  $N^{14}$ -ethanolamine is converted into  $N^{14}$ -choline to the same degree during the same period in adult rats fed on a diet deficient in labile methyl as in rats which were supplied with betaine as a donor of labile methyl. STETTEN concludes that the conversion of ethanolamine to choline proceeds without hindrance even when the diet is sufficiently poor in methyl groups to produce fatty livers. It is worth pointing out, however, that STETTEN employed an experimental period (10 days) which is when adult rats are used probably too short for definite conclusions to be made on the point in question. The reserves of labile methyl in adult rats are not likely to be markedly reduced in 10 days. In addition STETTEN's analyses seem to have been performed on the whole carcass and the result might therefore not necessarily indicate the conditions in the liver.

An increased phosphatide formation by the available choline might be brought about in at least two ways:

1) the choline given might go as such into the phosphatide formed.

2) it might donate its methyl groups to cephalin or to any compound which serves to methylate cephalin.

WELCH and LANDAU (1942) in experiments with arsено-choline have produced strong evidence for the fact that the former mode

of choline action normally proceeds in the rat liver. This does not exclude that the second mode may also be of importance. While it has been shown, however, that ethanolamine is easily methylated to choline in the rat organism (STETTEN 1941), it has not yet actually been demonstrated that this happens when the ethanolamine is bound in the cephalin molecule.

Concerning the cause of cephalin increase in the livers of the choline-deficient rats, only some suggestions can be advanced. (Of other investigators only STETTEN and GRAIL have found a similar rise of the cephalins without, however, making any comment to their findings). If the main process for the formation of lecithin in the choline-deficient animals were the methylation of cephalin, the accumulation of this latter compound might be conceived as an indication of a lowered rate of lecithin synthesis. It might also possibly be regarded as a regulative reaction furthering the lecithin synthesis. — Such explanations would also imply that the cephalins as such play very little or no part in the transfer of neutral fat by the liver. In fact neither ethanolamine nor cephalin displays the lipotropic action of choline or lecithin. The cause of this is not clear. The conception that the cephalins are structural phospholipids, as distinct from the lecithins which are metabolic ones can no longer be upheld, since it has been shown with the aid of isotopes that the metabolism of the cephalins in the liver is of about the same order as that of the lecithins.

According to HEVESY and HAHN (1941) a smaller part (about  $\frac{1}{4}$ ) of the liver cephalins has a more rapid metabolism than the lecithin, whereas the greater part has a much slower metabolism. (CHARGAFF (1942) suggests that these two forms of cephalin may be ethanolamine cephalin and serine cephalin). Furthermore SINCLAIR (1940) found that the rates of uptake of elaidic acid were practically the same for the lecithins and cephalins of the liver, although the maximum percentage of elaidic acid was significantly greater for the lecithins. In the present work it is seen how the cephalins during fasting decrease to quite the same extent as the lecithins in the liver. In alimentary lipidemia cephalin as well as lecithin, both probably deriving in the main from the liver (HEVESY and LUNDSGAARD 1937, HAHN and HEVESY 1938), are added to the blood, apparently in the same proportion in which they exist in the fasting blood, i. e. about 80 % lecithins to 20 % cephalins (BRANTE 1940). The difference

between lecithins and cephalins in the transfer of neutral fat by the liver may be quantitative rather than qualitative, the cephalins (the more metabolic part thereof) on a high fat diet not alone being able to prevent fat accumulation in the liver. It should also be considered that a relatively rapid metabolism of liver cephalin does not necessarily mean that it takes part as such in the transfer of neutral fat.

The cause of the increased osmotic resistance of the red blood corpuscles in the choline-deficient animals may be a lowered total phospholipid/cholesterol ratio in the corpuscles or a changed choline phospholipid/cephalin ratio. Chemical analyses should decide if either of these explanations is correct.

Evidence for the supposed rôle played by the adrenals in the choline or phospholipid metabolisms has not been provided by the present experiments. The influence of the adrenals on the neutral fat of the liver, which was demonstrated by MAC KAY and corroborated by the results of the present investigation, is therefore probably mediated by a mechanism which does not involve the phospholipids. BARNES, MILLER and BURR (1941) recently arrived at a similar conclusion when they found that while adrenalectomy decreased the rate of entrance of labelled fatty acids into the neutral liver fat, it did not change the normal rate of phosphorylation of absorbed fatty acids in the liver.

### Summary.

1. In adult rats fed for three to five months on a high fat diet practically free from choline, the fat content of the livers rose to about 25 % of the weight of fresh tissue. At the same time the content of choline phospholipids of the liver remained quantitatively unchanged as compared with rats given 5 mg choline per gm basic diet. The cephalins of the liver, on the other hand, showed a significant increase.

2. The red blood corpuscles of the same choline-deficient rats showed an increased resistance towards hypotonic NaCl and KCl solutions.

3. The neutral fat accumulated during the choline deficiency period disappeared in fasting. At the same time the phospholipid content of the liver markedly decreased, while the same choline phospholipid/cephalin ratio was maintained as existed before the beginning of the fast.

4. Adrenalectomy accelerated the disappearance of the accumulated neutral fat but did not produce any significant changes in the phospholipids in the liver.

5. In the kidneys, the spleen and the muscles neither choline deficiency, adrenalectomy nor fasting brought about any appreciable changes in the phospholipids.

6. Possible explanations for the results are discussed, especially the rôle of the different phospholipids in the transfer of neutral fat by the liver.

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# Über eine fällungstitrimetrische Mikromethode zur schnellen Bestimmung des Cholesterins.

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Eingegangen am 19. Juli 1943.

Zur quantitativen Bestimmung des Cholesterins stehen uns heute eine Reihe Methoden zur Verfügung, die teils auf kolorimetrischer, teils auf chemisch-analytischer Grundlage beruhen. Die kolorimetrischen Verfahren gründen sich fast ausschliesslich auf die bekannte Farbreaktion nach LIEBERMANN-BURKHARD und sind in vielen Modifikationen in der Literatur beschrieben. Die Reaktion selbst ist zwar sehr empfindlich, aber nicht genügend spezifisch, weshalb bei der direkten kolorimetrischen Bestimmung unbekannte Substanzen mitbestimmt werden können. Durch mehrere Arbeiten (LIFSHÜTZ 1913, MUELLER 1916, BÜRGER und REINHARDT 1919, MÜHLBOCK und KAUFMANN 1931) ist gezeigt worden, dass die bei klinischen Untersuchungen üblichen direkten kolorimetrischen Verfahren viel zu hohe Werte ergeben. Die zuverlässigste Ausführungsform ist die in neuerer Zeit von SCHÖNHEIMER und SPERRY (1934) entwickelte Methode, nach welcher das im Digitoninniederschlag befindliche Cholesterin kolorimetrisch bestimmt wird. Diese Methode hat wiederum verschiedene Abänderungen erfahren (SMITH und MARBLE 1937, SPERRY 1937).

Zur genauen Bestimmung des Cholesterins wird man sich im allgemeinen der weit zuverlässigeren Digitoninmethoden bedienen. Diese Bestimmungsweise geht auf WINDAUS (1910) zurück und gründet sich auf die von ihm gemachte Beobachtung, dass das Cholesterin mit Digitonin eine schwerlösliche Molekülverbindung gibt. Da nur das freie Cholesterin in Reaktion tritt, nicht aber di-Cholesterinester, ist hiermit die Möglichkeit gegeben, beide Fore

men getrennt zu bestimmen. Man hat sich vielfach um die Vereinfachung und Verbesserung des WINDAUS'schen Verfahren bemüht und dabei auch verschiedentlich versucht, die unbequeme gravimetrische Bestimmungsform durch andere Verfahren zu ersetzen. Zahlreiche Modifikationen sind vorgeschlagen worden, doch unterscheiden sich die Mehrzahl derselben nur wenig von einander. Hier sollen nur einige der wichtigsten Methoden und Abänderungen erwähnt werden.

Die gravimetrische Mikromethode ist zuerst von SZENT-GYÖRGYI (1923) ausgearbeitet und in neuerer Zeit von MANCKE (1930) sowie von MÜHLBOCK und KAUFMANN (1931) zu genauer Bestimmungsmethode ausgebaut worden. Auch eine titrimetrische Mikromethode ist von SZENT-GYÖRGYI (1923) entwickelt worden; sie beruht auf der Oxydation des Cholesterindigitonids mit einem Überschuss von Chromschwefelsäure und nachfolgender jodometrischer Bestimmung der überschüssigen Chromsäure. Diese Methode hat später zahlreiche Abänderungen erfahren; hier seien nur die Ausführungsformen nach OKEY (1930) und nach TURNER (1931) erwähnt. Einen prinzipiell anderen Weg zur titrimetrischen Cholesterinbestimmung haben RAPPAPORT und KLAPHOLZ (1933) vorgeschlagen. Sie füllen das Cholesterin mit einer bekannten überschüssigen Menge Digitonin und bestimmen im Filtrat der Fällung das überschüssige Digitonin, indem sie die abgespaltene Hexose durch die Methode nach HAGEDORN-JENSEN ermitteln. Schliesslich sei noch eine nephelometrische Bestimmungsmethode nach MÜHLBOCK, KAUFMANN und WOLFF (1932) erwähnt, nach welcher der Trübungsgang einer unter bestimmten Bedingungen ausgefüllten Digitonid-Suspension im Stufenphotometer gemessen wird. Durch Vergleich mit einer vorher festgelegten Eichkurve lässt sich die Cholesterinmenge berechnen.

Unter den verschiedenen Digitoninmethoden interessieren besonders die titrimetrischen Ausführungsformen, denn sie sind mit einfachen analytischen Hilfsmitteln ausführbar und erfordern keine kostspielige Apparatur. Die bisherigen Versuche, eine titrimetrische Bestimmungsmethode zu schaffen, haben jedoch zu einer Reihe Verfahren geführt, die ausnahmslos sehr umständlich und kompliziert sind. In erster Linie gilt dies einer Gruppe sehr ähnlicher Verfahren, die auf die erwähnte Methode von SZENT-GYÖRGYI zurückgehen, und die in grossen Zügen folgendermassen durchgeführt werden. 1. Filtration bzw. Zentrifugieren des aus-

gefällten Digitonids, 2. Auswaschen des Niederschlages mit Äther, Aceton, Chloroform o. dgl. zwecks Entfernung der Begleitstoffe, wie Fette und Lipoide, 3. Auswaschen des Niederschlages mit Alkohol und warmem Wasser zwecks Entfernung des überschüssigen Digitonins, 4. Oxydation des Digitonids mit Chromschwefelsäure (bzw. Silberchromatschwefelsäure) und nachfolgende jodometrische Bestimmung der überschüssigen Chromsäure. — Es ist einleuchtend, dass ein so kompliziertes Arbeitsverfahren den Forderungen an eine praktische Analysemethode nicht entsprechen kann. Die zahlreichen Auswaschungen des Niederschlages mit verschiedenen Lösungsmitteln sind besonders zeitraubend und in der Regel auch mit Schwierigkeiten verbunden. Während das Auswaschen mit den Fettlösungsmitteln im allgemeinen noch gut gelingt, werden sich bei dem Auswaschen mit Wasser leicht Suspensionen von Digitonid bilden, die schwer zu filtrieren bzw. zu zentrifugieren sind. Man läuft hierbei einerseits die Gefahr, dass das Digitonin nicht vollständig entfernt und somit bei der nachträglichen Oxydation in die Bestimmung miteinbezogen wird, andererseits dass wiederholtes Auswaschen mit Wasser bis zur restlosen Entfernung des Digitonins erhebliche Verluste an Substanz bedingen kann. Die hierbei entstehenden Fehler können zu völlig falschen Ergebnissen führen; jedenfalls muss man bei diesen Verfahren mit einer beträchtlichen Fehlerbreite rechnen.

Auch die titrimetrische Methode nach RAPPAPORT und KLAPOHLZ (1933) ist sehr kompliziert und zeitraubend, sie ist aber bemerkenswert dadurch, dass sie den ersten Versuch bildet, die praktischen Schwierigkeiten zu umgehen, die mit der quantitativen Isolierung des Cholesterindigitonids verbunden sind. Die hier benutzte indirekte Titration bedeutet wenigstens eine prinzipielle Vereinfachung, wenn auch der praktische Wert der Methode durch die umständliche »Rücktitration« sehr beeinträchtigt wird.

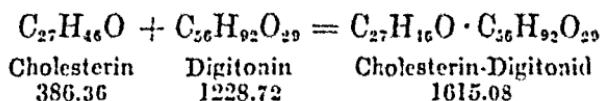
Die Prüfungsergebnisse der bisher bekannten titrimetrischen Digitoninverfahren veranlassten mich dazu, nach einer neuen Bestimmungsmethode zu suchen, die auf einfachere Weise und ohne erheblichen Zeitaufwand die Ausführung zuverlässiger Cholesterinbestimmungen gestattet.

### Prinzip der Methode.

In Anbetracht der Tatsache, dass das Cholesterin mit Digitonin eine schwerlösliche und recht stabile Molekülverbindung liefert,

war es bei der Suche nach einer einfachen und bequemen Bestimmungsmethode vor allem naheliegend, die Möglichkeit einer titrimetrischen Fällung in Erwägung zu ziehen. Eine Cholesterinbestimmung nach diesem Prinzip — insbesondere eine *direkte* Titration mit einer bekannten Digitoninlösung — würde sich offenbar viel einfacher als die bisherigen Digitoninmethoden gestalten, denn in erster Linie erübrigen sich hierbei die umständlichen und zeitraubenden Operationen, die zur Abscheidung und Isolierung des reinen Digitonids erforderlich sind. Von besonderer Wichtigkeit ist es auch, dass eine Reihe methodischer Fehler, die den bisherigen Digitoninverfahren anhaften — in erster Linie die Fehler, die durch das wiederholte Auswaschen des Digitonids mit verschiedenen Lösungsmitteln bedingt sind — bei einer titrimetrischen Fällung überhaupt nicht in Betracht kommen werden.

Ich stellte mir daher die Frage, ob die Reaktion zwischen Cholesterin und Digitonin nach der Gleichung:



zu einer massanalytischen Bestimmung verwertet werden könne.

Für eine solche Anwendung der Reaktionsgleichung müssen folgende Vorbedingungen erfüllt sein:

1. muss die Reaktion praktisch quantitativ nach der stöchiometrischen Gleichung verlaufen, und
2. muss sich der Titrationsendpunkt auf irgendeine Weise kennzeichnen lassen.

### Das Lösungsmittel.

Eine vollständige Umsetzung nach der stöchiometrischen Gleichung kann nur dann erzielt werden, wenn das Löslichkeitprodukt der Additionsverbindung einen hinreichend geringen Wert hat. Die erste Vorbedingung ist daher mit der Löslichkeit des Cholesterindigitonids und damit auch mit den Lösungseigenschaften des Reaktionsmilieus eng verknüpft.

Nach der ursprünglichen Methode von WINDAUS wird das Cholesterin in 95 proz. Alkohol gelöst und dann mit einer Lösung von Digitonin in 90 proz. Alkohol versetzt. Diese Arbeitsweise ist bei den später entwickelten Digitoninverfahren ohne wesentliche Änderungen beibehalten, in dem man gewöhnlich beide Sub-

stanzen in hochprozentigem Alkohol löst. Das Cholesterindigitonid ist aber, wie schon WINDAUS erkannte, in nicht unwesentlichem Masse in Alkohol löslich und zwar beträgt die Löslichkeit bei 18° 0.014 g, bei 25° 0.020 g in 100 ml 95 proz. Alkohol (WINDAUS 1910, SCHOENHEIMER und DAM 1933). Das Löslichkeitsprodukt der Additionsverbindung [Cholesterin] [Digitonin] hat demgemäß in alkoholischer Lösung einen so beträchtlichen Wert, dass die Reaktion nach den stöchiometrischen Verhältnissen nie eine vollständige sein wird. Um die quantitative Ausfällung des Cholesterins in alkoholischer Lösung zu erreichen, muss man deshalb einen reichlichen Überschuss an Digitonin verwenden, wie es auch bei den verschiedenen Verfahren die Regel ist. Die hier erwähnten Tatsachen machen es aber ohne weiteres klar, dass die Anwendung von Alkohol als Lösungsmittel bei der titrimetrischen Fällung des Cholesterins nicht zum Ziel führen wird.

In wässrigem Alkohol ist das Cholesterindigitonid bedeutend weniger löslich und zwar nimmt die Löslichkeit mit steigendem Wassergehalt stark ab. Die Anwendung eines solchen Lösungsmittels war aber ausgeschlossen, da das Cholesterin, wie auch die übrigen Lipoide, in wässrigem Alkohol sehr wenig löslich ist. In Aceton, Äther und Benzol ist das Digitonid so gut wie unlöslich und diese Flüssigkeiten sind außerdem vorzügliche Lipoid- und Fettlösungsmittel. Die unmittelbare Anwendung dieser Lösungsmittel für die Fällungtitration würde aber ebensowenig zum Ziel führen, da sie andererseits für das Digitonin kein Lösungsvermögen besitzen.

Cholesterin und Digitonin zeigen überhaupt sehr unterschiedliche Löslichkeitsverhältnisse und aus diesem Grunde wurde die Möglichkeit untersucht, zwei verschiedene, mit einander mischbaren Lösungsmittel bei der Fällungtitration zu verwenden: das eine zur Bereitung der als Massflüssigkeit dienenden Lösung von Digitonin, das andere zum Auflösen der zu bestimmenden Menge Cholesterin.

Als Lösungsmittel für das Digitonin erwies sich ein wässriger Alkohol am besten geeignet, doch musste die Alkoholkonzentration zwecks Herabsetzung der Löslichkeit des Cholesterindigitonids hinreichend gering gewählt werden. Durch Löslichkeitsbestimmungen bei verschiedenen Konzentrationen konnte festgestellt werden, dass das Cholesterindigitonid schon in 50 proz. Alkohol so gut wie unlöslich ist. In dieser Flüssigkeit ist das Digitonin genügend löslich (0.829 g in 100 ml bei 22° (LETTRÉ und

INHOFFEN 1936)) und die Lösung zeigte sich sehr gut haltbar, im Gegensatz zu Lösungen in 40 bzw. 30 proz. Alkohol, die nicht stabil waren. Die Bereitung der Massflüssigkeit soll weiter unten beschrieben werden.

Als Lösungsmittel für das Cholesterin konnte weiterhin Aceton verwendet werden, denn diese Flüssigkeit ist sowohl mit Wasser wie mit Alkohol in jedem Verhältnis mischbar und besitzt ausserdem, wie bereits erwähnt, für das Digitonid sehr geringes Lösungsvermögen.

Bei der Titration einer Lösung von Cholesterin in Aceton mit einer wässrig-alkoholischen Digitoninlösung wird sich die Fällungsreaktion somit im Lösungsmittelsystem Aceton-Wasser-Alkohol abspielen. Die quantitative Zusammensetzung dieses Gemisches wird sich natürlich während der Titration je nach dem Verbrauch an Massflüssigkeit ändern, doch konnte nachgewiesen werden, dass selbst ziemlich grosse Änderungen im Mischungsverhältnis Aceton: Wasser-Alkohol ohne Einfluss auf die Schwerlöslichkeit des Niederschlages waren. Das Digitonid erwies sich in allen Fällen so gut wie unlöslich und die Reaktion wird demnach im System Aceton-Wasser-Alkohol praktisch vollständig nach der stöchiometrischen Gleichung verlaufen.

Beim Mischen der zwei Lösungen, Cholesterin in Aceton und Digitonin in 50 proz. Alkohol, mussten andererseits auch die respektiven Löslichkeiten der Reaktionsteilnehmer im System Aceton-Wasser-Alkohol berücksichtigt werden, denn es ist natürlich die Voraussetzung, dass vorhandene Überschüsse sei es an Cholesterin oder an Digitonin bei jeder beliebigen Zusammensetzung des Gemisches stabil in Lösung bleiben. Dass das Lösungsmittelgemisch auch in dieser Beziehung den Anforderungen entspricht, konnte durch eine Reihe Mischungsversuche festgestellt werden. Hier sollen nur die folgenden zwei Versuche angeführt werden, die für die später angegebene Titrationsvorschrift von Interesse sind.

a) Eine Lösung von 2 mg Cholesterin in 2 ml Aceton wurde mit 2 ml 50 proz. Alkohol allmählich versetzt. Es entstand keine Trübung und auch nach 24 Stunden war das Cholesterin stabil in Lösung.

b) 2 ml Aceton wurden mit einer Lösung von 6.3 mg Digitonin in 2 ml 50 proz. Alkohol allmählich versetzt. Das Digitonin wurde hierbei nicht ausgefällt und auch nach 24 Stunden erwies sich die Lösung völlig klar.

### Die Bestimmung des Titrationsendpunktes.

Da kein geeigneter Indikator für die hier zu verwendende Fällungsreaktion zur Verfügung stand, wurde das altbekannte Prinzip herangezogen, die Masslösung so lange hinzuzugeben, bis ein weiterer Zusatz in der jedesmal geklärten Flüssigkeit keinen Niederschlag mehr erzeugt. Vollkommen richtige Werte liefert dieses Verfahren nicht, denn eine gesättigte Lösung von Digitonid wird sowohl mit Cholesterin- wie mit Digitoninlösung noch eine geringe Fällung zeigen. Titriert man daher die Cholesterinlösung so lange, bis ein weiterer Zusatz von Digitonin keine Trübung mehr hervorruft, so geht man etwas über den eigentlichen Endpunkt hinaus. Dieser Fehler kann jedoch kompensiert werden, wenn die Bestimmung unter den gleichen Bedingungen erfolgt, wie die Herstellung der Digitoninlösung selbst auf einen bekannten Cholesterinstandard.

Wegen der besonderen Eigenschaften des ausgefällten Cholesterindigitonids lässt sich die Titrationsflüssigkeit durch einfaches Absitzenlassen des Niederschlages nicht genügend schnell und vollständig klären, auch nicht, wenn der letztere durch Erwärmen in kristallinische Form überführt wird. Die Beseitigung des kristallinisch abgeschiedenen Niederschlages kann aber schnell und vollständig durch Zentrifugieren erzielt werden und es erwies sich daher zweckmäßig, die Titrationen direkt in kleinen Zentrifugen-gläsern vorzunehmen. In der so geklärten Flüssigkeit lässt sich der Titrationsendpunkt ziemlich genau bestimmen, indem noch Mengen von 0.01 bis 0.02 mg Cholesterin eine deutliche Trübung hervorrufen werden.

### Bereitung der Masslösung.

Aus praktischen Gründen wird mit einer empirischen Digitoninlösung gearbeitet: 1 ml derselben soll möglichst genau 1.00 mg Cholesterin entsprechen und soll somit eine dieser Menge äquivalente Digitoninmenge, also  $1.00 \cdot \frac{1228.72}{386.36} = 3.18$  mg gelöst enthalten.

Die Digitoninpräparate sind selten ganz rein; man bereitet daher die Masslösung durch Auflösen von 0.32—0.33 g Digitonin cryst. Merck (bei 100° getrocknet) in 100 ml wässrigem Alkohol

von 50 % und filtriert die etwa unklare Flüssigkeit nach ein oder zwei Tagen. Die so bereitete Lösung ist sehr lange haltbar.

Um den genauen Umrechnungsfaktor sicherzustellen, wird die Digitoninlösung auf einen bekannten Cholesterinstandard eingestellt.

### Ausführung der Titration.

Mit der hier zu verwendenden Endpunktsbestimmung wird es natürlich unpraktisch sein, die Titration in der üblichen Weise auszuführen, denn ein solches Verfahren würde des häufigen Zentrifugierens wegen zu umständlich und zeitraubend sein. Die Bestimmung wird sich erheblich einfacher gestalten, wenn man zunächst durch eine Vortitration einen Näherungswert für den gesuchten Verbrauch ermittelt. Die Masslösung wird zu diesem Zweck in grösseren Anteilen zugesetzt und man legt den Endpunkt innerhalb etwa 0.200 ml fest. Bei einer zweiten Titration wird von vornherein fast die gesamte zur Fällung erforderliche Menge Masslösung zugesetzt und der Endpunkt schärfer ermittelt. Zur Kontrolle wird eine dritte Bestimmung ausgeführt.

Von der zu untersuchenden Lösung von Cholesterin in Aceton, die nicht mehr als etwa 1 mg in 1 ml enthalten darf, werden 2 ml in einem Zentrifugengläschen abpipettiert. (Man verwendet hierfür Gläschen mit schmal ausgezogenen Boden und von etwa 15 ml Inhalt. Sie müssen zuvor gründlich, am besten in Chromschwefelsäure gereinigt sein.) Man versetzt die Probe aus einer Mikrobürette (5 ml Inhalt, mit 0.01 ml Teilung) mit beispielsweise 0.400 ml Masslösung, wobei das Cholesterindigitonid fast augenblicklich als milchiger, sehr voluminöser Niederschlag ausfällt. Das Gläschen wird in ein Wasserbad von etwa 65° gebracht, indem man zunächst nur 1—2 cm des unteren Endes in das Wasser eintauchen lässt. Durch die hierbei erzeugte Wärmebewegung kommt die Flüssigkeit in lebhafter Zirkulation und das Digitonid scheidet sich nach wenigen Minuten in kristallinischen Flocken ab. Das Gläschen wird dann etwas tiefer, etwa bis zur Hälfte, in das Wasserbad versenkt und weiter erwärmt, bis der kristallinische Niederschlag sich von der vollkommen klar gewordenen Flüssigkeit scharf abgegrenzt hat. Die Probe wird nun aus dem Wasserbade entfernt, mit kaltem Wasser abgekühlt und 3 Minuten zentrifugiert (2,000 bis 2,500 Touren pro Minute). Zu der vollkommen klaren Flüssigkeit gibt man nun genau 0.200 ml Masslösung hinzu und beobachtet, ob eine weitere Fällung von Digitonid entsteht.

Ist dies der Fall, wird die Probe noch kurze Zeit im Wasserbade erwärmt und nachher 3 Minuten zentrifugiert. Auf diese Weise fährt man fort, bis ein weiterer Zusatz von 0.200 ml Masslösung keinen Niederschlag mehr erzeugt. Die Auswertung dieser Vortitration geht aus dem folgenden Beispiel hervor:

Zugabe von Masslösung:	Fällung:	Gesuchter Verbrauch:
0.400 ml	+	
+ 0.200 »	+	Mehr als 0.400 ml
+ 0.200 »	+	» » 0.600 »
+ 0.200 »	+	» » 0.800 »
+ 0.200 »	—	Weniger als 1.000 »

Der gesuchte Verbrauch liegt hiernach zwischen den Grenzwerten 0.800—1.000 ml. In einer zweiten Probe der Untersuchungslösung (2 ml) fällt man daher das Cholesterin direkt mit 0.800 ml Masslösung, scheidet das Digitonid in der oben beschriebenen Weise ab und zentrifugiert 3 Minuten. Hat man auf diese Weise die Hauptmenge des Cholesterins ausgefällt und das Digitonid aus der Lösung beseitigt, kann man schliesslich den noch vorhandenen Überschuss austitrieren und somit den Titrationsendpunkt genau ermitteln. Bei der Endtitration darf jedesmal nur 0.03 bis 0.05 ml hinzugefügt werden und die kleinen Mengen Digitonid, die hierbei eventuell entstehen, können ohne vorausgehende Erwärmung auszentrifugiert werden, da sie sich schon bei gewöhnlicher Temperatur nach wenigen Sekunden kristallinisch abscheiden. In unmittelbarer Nähe des Endpunktes wird man die Masslösung nur tropfenweise hinzufügen; die Trübung entsteht hierbei nicht momentan, sondern erst nach einigen Sekunden und zwar wird sie dann zuerst im unteren Teil der klaren Flüssigkeit in Erscheinung treten. Die Gläser werden am besten in reflektiertem Licht beobachtet, bei künstlicher Belichtung empfiehlt es sich außerdem, auf einer Unterlage von schwarzem Papier zu arbeiten.

Die gesuchte Menge Cholesterin in Milligram ergibt sich direkt aus der verbrauchten Anzahl Milliliter Masslösung, bzw. durch Multiplikation mit dem bei der Titerstellung genau ermittelten Umrechnungsfaktor.

Im einzelnen sei zu dem Verfahren noch folgendes bemerkt. Für die eindeutige Feststellung der Trübungen ist es von Wichtigkeit, dass die Gläschchen vollkommen klar und sauber sind. Unter Umständen kann eine dünne Schicht aus Digitonidkristallen an

den Glaswänden haften und dadurch eine sichere Beobachtung schwierig machen. Dieser Übelstand kann mitunter durch mangelnde Sauberkeit der Gläser verursacht werden, dürfte aber in den meisten Fällen eine Folge ungenügender Erwärmung sein. Die Abscheidung des Digitonids als kristallinischer Niederschlag muss daher sehr sorgfältig vorgenommen werden, um den erwähnten Fehler zu vermeiden. Je grössere Mengen Digitonid, desto länger muss die Erwärmung im Wasserbade dauern, doch wird in der Regel eine Erwärmungsdauer von 5 bis 10 Minuten ausreichend sein.

### Versuchsteil.

#### Titerstellung der Digitoninlösung.

Bei der Titerstellung verfährt man prinzipiell in der gleichen Weise wie bei der Titration einer unbekannten Cholesterinlösung, doch wird man hier die Hauptmenge des Cholesterins unmittelbar aussäubern können, da die zu bestimmende Menge von vornherein gegeben ist.

Zur Herstellung des Cholesterinstandards wurden 73,5 mg reines, trockenes Cholesterin (Schmp. 148°) in einem 100-ml-Messkolben in reinstem Aceton gelöst und bis zur Marke aufgefüllt. 2 ml dieser Lösung entsprachen 1.470 mg Cholesterin und der voraussichtliche Verbrauch an Masslösung würde somit etwa 1.47 ml betragen. Zwei Proben von je 2 ml, in etwa gleich schweren Zentrifugengläschchen abpipettiert, wurden daher direkt mit je 1.400 ml Masslösung versetzt, die Niederschläge in der angegebenen Weise abgeschieden und dann 3 Minuten zentrifugiert. Das noch in den Lösungen befindliche Cholesterin wurde schliesslich tropfenweise austitriert. Als endgültige Titrationswerte ergaben sich dabei 1.459 und 1.464 ml, im Mittel 1.462 ml. Der Titer ergab sich hierauf zu: 1.470 : 1.462 = 1.005, d. h. 1 ml der Masslösung entsprach 1.005 oder rund 1.00 mg Cholesterin.

#### Bestimmung des freien Cholesterins im Blut.

Die Extraktion erfolgt nach Bloor. 10 ml Plasma oder Oxalatblut werden tropfenweise unter Schütteln zu 200 ml Alkoholäther (3 : 1) gegeben, man lässt einige Stunden stehen, filtriert durch ein Faltenfilter und wäscht mit Alkoholäther nach. Das Filtrat wird vollständig eingedampft, der Rückstand in reinem Aceton aufgenommen und die Lösung filtriert. Die Acetonlösung wird eingeengt und im Messkolben auf 25 ml aufgefüllt. 2 ml dieser Lösung entsprechen 0.80 ml Plasma oder Blut.

Beispiel 1. — Aus dem nach dieser Vorschrift bereiteten Lipoidextrakt wurden drei Proben von je 2 ml in Zentrifugengläschchen abpipettiert. Die Vortitration ergab zunächst, dass der Verbrauch zwischen den Grenzen 0.400—0.600 ml lag. Die zwei übrigen Proben wurden demgemäß mit je 0.400 ml Masslösung gefällt, im Wasserbade

behandelt und die Niederschläge auszentrifugiert. Die nachträgliche Titration ergab als endgültige Werte 0.532 und 0.524 ml, im Mittel 0.528 ml, entsprechend  $0.528 \cdot 1.005 = 0.531$  mg Cholesterin. Diese Menge war in 0.80 ml Blut enthalten; der Gehalt an freiem Cholesterin betrug daher 66.4 mg %.

### Bestimmung des Gesamtcholesterins im Blut.

Da das Cholesterin des Blutes teils in freier teils in gebundener Form vorliegt, muss zur Bestimmung des Gesamtcholesterins eine Verseifung des Blutes vorausgehen. Zur Herstellung des Lipoideextraktes wurde folgende Arbeitsweise verwendet:

5 ml Serum wurden mit 5 ml 10 prozentiger alkoholischer Kalilauge 1 Stunde auf dem Wasserbade unter öfterem Umschwenken verseift (Rückflusskühler oder Steigrohr). Nach Verdünnen mit 10 ml Alkohol und 50 ml Wasser wurde die Seifenlösung in einen etwa 200 ml fassenden Scheidetrichter überführt und mit 30 ml Äther versetzt. Der Scheidetrichter wurde kräftig geschüttelt und stehengelassen, bis die beiden Flüssigkeitsschichten sich getrennt und geklärt hatten. Die Seifenlösung wurde in einen anderen Scheidetrichter abgelassen und noch zweimal, jedesmal mit 30 ml Äther in der gleichen Weise ausgezogen; die Ätherauszüge wurden in den ersten Scheidetrichter überführt. Die vereinigten Ätherauszüge wurden dreimal mit je 30 ml Wasser zwecks Entfernung von Alkohol und Seife geschüttelt; bei der letzten Auswaschung wurde das Wasser mit 1 ml n/10 Salzsäure angesäuert. Der Ätherextrait wurde in einen Rundkolben gebracht und der Äther auf dem Wasserbade abdestilliert. Der Rückstand wurde in etwa 10 ml Aceton gelöst und unter Nachspülen mit Aceton in einen 25-ml-Messkolben überführt; dann wurde bis zur Marke aufgefüllt. 2 ml dieser Lösung entsprachen 0.40 ml Serum.

Beispiel 2. — Aus der nach dieser Vorschrift bereiteten Cholesterinlösung wurden drei Proben von je 2 ml in Zentrifugengläschen abpipettiert. Die Vortitration ergab die Grenzwerte 0.800—1.000 ml, weshalb die zwei übrigen Proben direkt mit je 0.800 ml Masslösung gefällt wurden. Nach Abscheidung der Niederschläge und Zentrifugieren ergaben die Titrationen als endgültige Werte 0.848 und 0.860 ml, im mittel 0.854 ml, entsprechend  $0.854 \cdot 1.005 = 0.858$  mg Cholesterin. Da diese Menge in 0.40 ml Serum enthalten war, betrug der Gesamtcholesteringehalt: 214 mg %.

Als ständige Begleiter des Cholesterins sind auch die Provitamine Ergosterin und 7-Dehydro-Cholesterin im Blut vorhanden. Da sie ebenfalls mit Digitonin fällbar sind, werden sie in die Bestimmung miteinbezogen. Ihre Menge ist aber sehr gering (WINDAUS 1936) und liegt in allen Fällen innerhalb der Fehlergrenzen der Bestimmung. Mit Digitonin fällbar sind ferner einige Substanzen, die der Gruppe der Sexualhormone gehören (trans-Dehydroandrosteron,  $\alpha$ -Östradiol). Auch diese Substanzen sind in so geringer Menge im Blut vorhanden, dass sie den wahren Cholesterinwerten nicht merkbar beeinflussen werden.

### Die Analysenfehler.

Wie schon erwähnt, wird der eigentliche Titrierfehler (Indikatorfehler) bei der hier beschriebenen Methode praktisch ausgeschaltet, da die Titration der Untersuchungslösung unter den gleichen Bedingungen erfolgt, wie die Titerstellung der Masslösung gegen den Cholesterinstandard. Durch diese Massnahme lassen sich auch andere methodische Fehler weitgehend eliminieren, weshalb die Genauigkeit der Ergebnisse vorwiegend von den allgemeinen Titrierfehlern bestimmt wird (Tropfenfehler, Nachlauf- und Ablesefehler usw.). Um den Einfluss dieser Fehler unmittelbar feststellen zu können, wurde eine grössere Anzahl Bestimmungen in einer genau bekannten Cholesterinlösung ausgeführt. Es wurde hierfür eine Lösung verwendet, die 1.000 mg Cholesterin in 2 ml enthielt. Die Ergebnisse der Analysen sind in der folgenden Tabelle wiedergegeben.

	Verbrauch A ml	mg Cholesterin $\approx A \cdot 1.005$	Abweichung f vom Mittelwert	$f^2$
1	0.991	0.996	-0.005	$0.25 \cdot 10^{-4}$
2	1.003	1.008	+0.007	0.49
3	1.008	1.013	+0.012	1.44
4	0.983	0.988	-0.013	1.69
5	1.000	1.005	+0.004	0.16
6	1.010	1.015	+0.014	1.96
7	0.989	0.994	-0.007	0.49
8	1.007	1.012	+0.011	1.21
9	0.985	0.990	-0.011	1.21
10	0.990	0.995	-0.006	0.36
11	0.986	0.991	-0.010	1.00
12	1.003	1.008	+0.007	0.49
13	0.992	0.997	-0.004	0.16
14	1.005	1.010	+0.009	0.81
Mittelwert: 1.001			$\Sigma(f^2) = 11.72 \cdot 10^{-4}$	

Der Mittelwert dieser Analysen fällt wie ersichtlich mit dem theoretischen Wert genau zusammen. Aus der Summe der Fehlerquadrate  $\Sigma(f^2)$  und der Anzahl Messungen können die verschiedenen Fehlergrössen ermittelt werden: Der mittlere Fehler der Einzelmessung  $f_m = \sqrt{\frac{11.72 \cdot 10^{-4}}{13}} = \pm 0.009$  mg; der mittlere Fehler

des Mittelwertes  $F_m = \sqrt{\frac{11.72 \cdot 10^{-4}}{14 \cdot 13}} = \pm 0.003$  mg. In der Regel

wird man nur zwei Parallelen bestimmen und von diesen das Mittel nehmen; der mittlere Fehler ergibt sich in diesem Falle zu:  $\pm 0.009 : \sqrt{2} = \pm 0.007$  mg. Damit dieser Fehler sich nicht zu stark geltend macht, sucht man nach Möglichkeit die Konzentra-

tion der Untersuchungslösung so einzustellen, dass die zu titrirende Cholesterinmenge nicht weniger als etwa 0.5 mg beträgt. Der relative Fehler bei der Titration braucht somit nicht etwa  $\pm 1.4\%$  zu übersteigen und wird im Gebiete 1.0—2.0 mg Cholesterin nur  $\pm 0.7$ — $0.4\%$  betragen. Bei der Titerstellung mit nur zwei Parallelen wird sich gleichfalls eine Ungenauigkeit von  $\pm 0.007$  ml einstellen; der relative Fehler wird aber hier nur etwa  $\pm 0.5\%$  betragen. Man wird demnach mit einer durchschnittlichen Fehlerbreite von  $\pm 1$ — $2\%$  rechnen und dürfte damit eine für gewöhnliche Cholesterinbestimmungen hinreichende Genauigkeit erreicht haben.

### Zusammenfassung.

Es wird ein neues Mikroverfahren beschrieben, nach welchem das Cholesterin durch direkte Titration mit einer bekannten Digitoninlösung bestimmt werden kann. Damit die Fällungsreaktion nach der stöchiometrischen Gleichung verlaufen soll, muss ein Lösungsmittel verwendet werden, in welchem das Cholesterindigitonid praktisch unlöslich ist. Hochprozentiger Alkohol, der gewöhnlich bei Digitoninfällungen zu Anwendung kommt, erfüllt bekanntlich diese Forderung nicht. Das zu bestimmende Cholesterin, das aus dem Untersuchungsmaterial in geeigneter Weise extrahiert ist, wird daher in Aceton gelöst und ein aliquoter Teil mit einer genau bekannten Lösung von Digitonin in 50 prozentigem Alkohol titriert. Die Fällungsreaktion wird sich hierbei im Lösungsmittelsystem Aceton-Wasser-Alkohol abspielen; die Löslichkeit des Digitonids in diesem Gemisch ist sehr gering und wird von der wechselnden Zusammensetzung desselben praktisch nicht beeinflusst.

Die Titrationen werden in kleinen Zentrifugengläsern ausgeführt und der Titrationsendpunkt lässt sich in der Wiese bestimmen, dass man von der Masslösung so lange zugibt, bis in der jedesmal durch Zentrifugieren geklärten Flüssigkeit keine Trübung mehr entsteht. Die Bestimmung wird sich am einfachsten gestalten, wenn man zunächst durch eine Vortitration einen Näherungswert für den gesuchten Verbrauch ermittelt. Bei einer zweiten Titration wird von vornherein fast die gesamte zur Fällung erforderliche Menge Masslösung zugesetzt und der Endpunkt schärfer ermittelt. Von der empirischen Masslösung soll 1 ml genau 1.00 mg Cholesterin entsprechen; sie wird auf einen be-

kannten Cholesterinstandard eingestellt. Die mittlere Fehlerbreite beträgt etwa  $\pm 1-2\%$ .

Die Cholesterinbestimmung im Blut wird an Hand zweier Analysenbeispiele beschrieben und für die Verseifung und Extraktion des Blutserums wird eine besondere Vorschrift gegeben. Die Methode ist sehr einfach und schnell ausführbar; sie dürfte sich deshalb zu klinischen Untersuchungszwecken gut eignen.

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## The Rôle of the Chemoreceptors of the Sinus Region for the Occlusion Test in the Cat.

By

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Received 19 August 1943.

As is well known, occlusion of both common carotids is followed by a rise in the general blood pressure, a quickening of the heart rate, and an increase of respiration. These effects on circulation have been generally attributed to a reduction of the number of impulses elicited from the baroceptors of the sinus region by the diminished intracarotid pressure. This will lead to a disinhibition of the vasoconstrictor and heart accelerator centres and a decreased function of the vagal centre (cf HEYMANS, BOUCKAERT and REGNIERS 1933).

A corresponding explanation was at first given for the effect on respiration. Thus it was assumed that a tonic reflex inhibition of respiration was normally exercised from the baroceptors of the sinus and that the clamping of the common carotids reduce or abolish this effect (HEYMANS and BOUCKAERT 1930).

With regard to respiration, it soon became evident, however, that the occlusion of the common carotids gives rise to a stimulation of the chemoreceptors of the carotid body, as was seen especially from the action potentials from the sinus nerve (cp EULER, LILJESTRAND and ZOTTERMAN 1939). This is undoubtedly the main factor leading to the increased respiration. No evidence has been adduced that respiration is in any way reflexly influenced from the baroceptors of the sinus or aortic regions under normal conditions (EULER and LILJESTRAND 1937), though an effect has been observed after section of the vagodepressor nerves (BJURSTEDT and EULER 1942 a).

The question whether the effect on circulation of clamping the common carotids may be due, not only to a decreased stimulation of the baroreceptors, but also to a stimulation of the chemoreceptors of the region has not, as far as we are aware, been discussed. It seems to be tacitly assumed that in this case the reflex from the baroreceptors alone is responsible. Against this view objections can be advanced, however. That a considerable hypoxia or hypercapnia through stimulation of the chemoreceptors of the sinus region may cause an increase in the general blood pressure, has been found by several authors (HEYMANS, BOUCKAERT, EULER and DAUTREBANDE 1932, EULER and LILJESTRAND 1936, WINDER, BERNTHAL and WEEKS 1938, BOUCKAERT, GRIMSON, HEYMANS and SAMAAN 1941, BJURSTEDT and EULER 1942 b). Recent experiments (EULER and LILJESTRAND 1942) have also made it clear that the arterial blood pressure and the pulse rate are influenced reflexly from the sinus region already by variations in the oxygen pressure within the physiological limits. Thus it was found that when oxygen was substituted for air in anesthetized cats during artificial respiration, the blood pressure and the pulse rate regularly showed a decrease; after denervation of the sinuses and section of the vagodepressor nerves, oxygen inhalation led to an increased blood pressure, and most of the action on the pulse rate disappeared. These results have recently been confirmed also for the rabbit (HEIJNEMAN 1943).

From the observations quoted it would seem probable that stimulation of the chemoreceptors in the sinus region during occlusion of the carotids may to some extent play a rôle as regards the increase in blood pressure. In order to elucidate this point the following experiments were carried out.

In cats in chloralose anesthesia both common carotids were exposed. Arterial blood pressure was recorded from the femoral artery, and artificial respiration was maintained at a constant level with a respiration pump, bags with gas mixtures being attached to it when such were to be used instead of air. The ventilation was just sufficient to suppress spontaneous respiratory movements, but overventilation, which quickly leads to a lowering of the blood pressure, was carefully avoided. After a preliminary period of 2 minutes, during which ventilation with the gas mixture was performed, the common carotids were closed with clamps for 1 minute, this being followed by another period of 2 minutes before the next gas mixture was tried.

Fig. 1 gives a typical experiment. The general level of the blood pressure was but little affected by the gas mixtures used, the

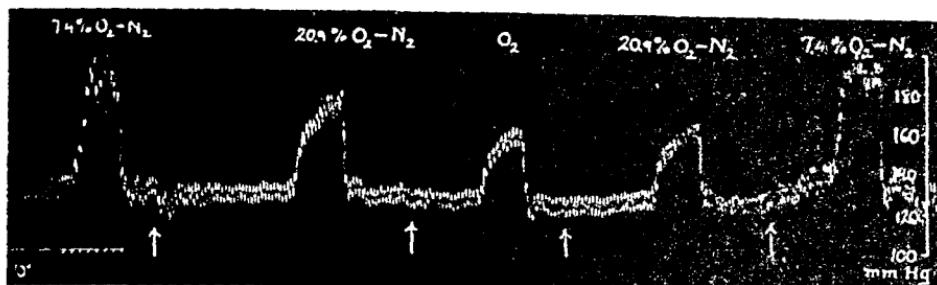


Fig. 1. Cat in chloralose anesthesia. Blood pressure from femoral artery. Vagodepressors intact. Constant artificial respiration with different gas mixtures (starting at the arrows). Effect of occlusion of both common carotids.

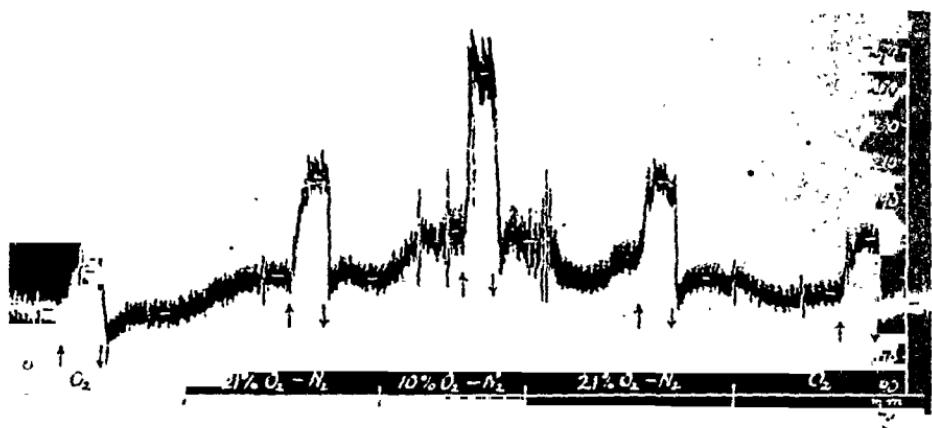


Fig. 2. Cat, 3.8 kg, chloralose. Blood pressure from femoral artery. Vagodepressors intact. Constant artificial respiration with gas mixtures as marked on the tracing. Between arrows occlusion of both common carotids. Time marking 10 sec.

average pressure values being 128, 127 and 132 mm respectively for oxygen, air and 7.4 % oxygen in nitrogen. But it is obvious that a greater response to the carotid occlusion test was obtained when the oxygen pressure in the inspired air was reduced. Already in air-breathing the occlusion response (an increase of 42 and 33 mm respectively, corresponding to 33 and 26 %) was distinctly greater than with oxygen (25 mm, i. e. 19 %), and it was considerably greater (60 and 62 mm, 47 % and 46 %), when the animal was breathing 7.4 % oxygen in nitrogen, which of course produced a marked hypoxia.

In fig. 2 another experiment is given, where, in contrast to the one just mentioned, the general blood pressure already before the occlusion was greatly influenced by the oxygen pressure. It started at about 135 mm during ventilation with oxygen, rose with air to about 150 and with 10 % oxygen in nitrogen

to 170. When air was given again, the pressure went down to 159 and with oxygen to 137 mm. The increase during the occlusion test was much more influenced by the oxygen pressure, however. During oxygen ventilation it was only 19 mm (14 %) in the first and 33 (24 %) in the last determination, with air the corresponding figures were respectively 51 (34 %) and 39 (34 %) and with 10 % oxygen in nitrogen 83 (49 %). The irregularities of the blood pressure tracing during the period of breathing 10 % oxygen in nitrogen is due to spontaneous breaths induced by the strong stimulus of oxygen want. The artificial ventilation was just adequate when the animal was breathing air.

It seems hardly probable that the decreased oxygen pressure in the arterial blood will lead to an increased excitability of the vasomotor centre. The evidence available points decidedly in the opposite direction (cf LAMBERT and GELLHORN 1938). We also made some direct experiments on this point, following the blood pressure response after a constant stimulation of the central end of the depressor nerves when the animal had been ventilated with oxygen, air and 10 % oxygen in nitrogen. No differences were observed.

The only satisfactory explanation that can be offered therefore seems to be that during the occlusion test the blood pressure rises partly in consequence of putting out of action the baroceptors and partly because the chemoreceptors are stimulated by oxygen want as a result of the reduced blood supply. It is of special interest that already during air breathing under the actual experimental conditions both factors are operating, though the effect from the baroceptors plays the greatest rôle.

### Summary.

The effects of occlusion of the common carotids on the general blood pressure in anesthetized cats is decreased when oxygen is substituted for air, but increased if the arterial oxygen tension is decreased by ventilating with 7—10 % oxygen in nitrogen instead of air. The blood pressure before occlusion is more or less influenced in the same direction.

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## Changes in Respiration when Changing to Different Postures.

By

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Received 31 August 1943.

Experiments with regard to the relations between posture and respiration in man have generally been carried out in lying, sitting and standing positions. It has been found that frequency is least when one is lying, greater when sitting and greatest when standing. It is the same thing with ventilation (cf. BOHR 1907, LILJESTRAND and WOLLIN 1913, HALDANE, MEAKINS and PRIESTLEY 1918). The alveolar carbon dioxide tension is accordingly lowest in a standing, greater in a sitting and greatest in a lying position (LILJESTRAND and WOLLIN 1913, HIGGINS 1914). SOLEY and SHOCK (1940) found that during the first ten minutes after taking up a lying position an average decrease of 40 % was obtained in the respiration volumes, and in the next ten minutes there was a further 10 % decrease. There was no considerable change in the following ten minutes. DONAL, GAMBLE and SHAW (1934) obtained in the above mentioned positions coinciding results with previous investigators, and in addition to this a considerable increase of frequency and respiration volume in the vertical head-down position.

MARK and NEUMANN (1931) used a revolving lounge chair which could be fixed at different angles, the extreme angle of 70° being used for the head-up position, and 60° for the head-down. The respiration was recorded by means of two pneumographs, one round the thorax, the other round the abdomen. When

changing to the head-up position they found in most cases a decrease in the breathing frequency, and when going over to the head-down there was an increase in frequency with a decrease in the depth of breathing. When making a rapid change from one position to another a temporary retardation and deepening of respiration was obtained throughout. The retardation of respiration when changing from the horizontal to the head-up position which MARK and NEUMANN have found in man is in good agreement with the results obtained from animal experiments. They presume that the increase in respiration when changing from a lying to a standing position, which other investigators have obtained, has to do with the appearance of active muscular work.

It has also been established that a retardation of respiration occurs when the head is bent far backwards, and when bent forwards respiration is hastened. This has been proved both in the case of man (MARK 1932) and animals (MARK 1933). In man when the head was down and bent backwards there was a retardation, but there was only an indication of this when the head was up. MARK (1932) points out the resemblance between the results with the different positions of the head and those obtained when pressing the carotid arteries and the carotid sinus respectively (MARK and NEUMANN 1931).

When experimenting with dogs CYBULSKI (1879) found that a retardation in respiration occurred when there was a passive change from the horizontal to the vertical head-up position, and that respiration was more frequent when the vertical position was reversed. SALATÉ had previously made the same observation (1876) and had moreover found (1877) that when rabbits were placed in the vertical head-up position, they died within 15 minutes to two hours. BLUMBERG (1885) found in cats and rabbits more frequent respiration and a decrease in frequency respectively under the conditions mentioned above, and by dividing the vagi he was able to neutralize this effect of the posture on the respiration. HILL (1895) like BLUMBERG found after dividing the vagi no decrease in respiration frequency when changing to the vertical head-up position. For recording HILL made use of a pneumograph round the thorax, and from his experiments as well as from previous investigations on animals it has only been possible to get a definite conception of the frequency and not of the volume of the ventilation.

### Method.

Experiments have been carried out on 8 rabbits besides one on cat. The rabbits were anaesthetized with urethane, 1.5 g pr kg body weight intravenously, except in one case when a mixture of urethane and chloralose was used. In the cat experiment 0.035 g pr kg body weight chloralose + 1 cc 20 % urethane solution was employed. In order to record the respiration an air-tight box — a so-called body plethysmograph — was used, and the animal was placed in this and fastened to a board with its abdomen upwards. The box was connected to a spirometer which recorded the respiration movements on kymograph paper. The animals breathed through a tracheal cannula connected with the air outside the box. The blood-pressure was recorded by means of a mercury manometer connected to the femoral artery. Before the experiment was begun both carotid arteries were exposed in five cases. In order to control that the sinus regions were intact the animals were first made to inhale a gas mixture containing about 5—10 % of oxygen in nitrogen which was followed by an increased respiration. The same method was afterwards applied in order to control the effectiveness of the denervation (see below). The box was changed from the horizontal to different positions, i. e. a vertical and a + 45° head-up position and a vertical and a — 45° head-down position. The two first mentioned positions will be called + 90° and + 45° respectively, and the head-down positions — 90° and — 45°. The experiments were repeated several times and in five cases experiments were made after the denervation of the two sinus regions. The rotation axis went through the cannula in the femoral artery. Two experiments were also made recording the respiration in the changes of position before and after the division of both vagi.

### Results.

*Rabbits.* When changing the animal from a horizontal position to a + 90° a marked decrease in ventilation was brought about, and sometimes respiration stopped entirely for a few seconds. A strong decrease in frequency was most often obtained as well as a pronounced decrease in amplitude. Ventilation subsequently increased to a certain extent. In most cases the animals were not kept in the new position for more than a few minutes and most often it was not so long as that. When the animal was afterwards put back into the horizontal position, respiration often became accelerated far beyond the normal, i. e. before the change to the vertical position (see fig. 1 A). After this the breathing decreased and slowly reverted to normal frequency and amplitude.

When changing the animal to a + 45° position, too, a decrease in ventilation was also brought about, though this was not so

considerable as when changing to + 90°. After returning to the horizontal position respiration became accelerated and it gradually went back to the normal.

After the denervation of the two sinus regions the same effect was obtained as has been described, i. e. a decrease in ventilation when changing to + 90° and + 45° as well as more than normally increased respiration after going back to the horizontal position. As will be seen from the table, there seems, however, to be a tendency towards a greater decrease in amplitude after denervation than with intact animals, while frequency does not seem to be influenced as much after denervation.

The table below gives the values from a typical experiment on a 2.1 kg rabbit.

The sinus regions intact	Breaths pr min.	Average breaths in ml.	Ventilation ml/min.	Ventilation in the new position in % of the ventilation in the horizontal position
Horizontal position <sup>1</sup> + 90° position <sup>2</sup>	63.2 41.7	15.50 11.48	980 479	48.9 %
Horizontal position <sup>1</sup> + 90° position <sup>2</sup>	62.0 41.2	14.68 12.24	910 504	55.4 %
Horizontal position <sup>1</sup> + 45° position <sup>2</sup>	67.6 59.8	14.64 14.10	990 843	85.2 %
Horizontal position <sup>1</sup> + 45° position <sup>2</sup>	65.5 58.4	14.68 14.16	962 827	86.0 %
The sinus regions denervated on both sides				
Horizontal position <sup>1</sup> + 90° position <sup>2</sup>	50.0 41.0	15.92 9.42	796 386	48.5 %
Horizontal position <sup>1</sup> + 90° position <sup>2</sup>	49.4 40.0	15.60 9.28	771 371	48.1 %
Horizontal position <sup>1</sup> + 45° position <sup>2</sup>	50.4 50.4	15.56 12.60	784 635	81.0 %
Horizontal position <sup>1</sup> + 45° position <sup>2</sup>	49.0 43.9	16.14 12.82	791 563	71.2 %

Similar results were obtained in the other experiments at these positions.

<sup>1</sup> Just before changing to next position.

<sup>2</sup> Calculated from the beginning ca 20 seconds after changing to position in question, ca 20 breaths onwards.

The effect is not so great when going to the head-down positions, — 90° and — 45°, as to the head-up. Frequency has shown varying conditions. In the — 45° with the sinus regions intact in the six animals on which the experiments were carried out, the frequency in three of them showed a considerable increase, while in the other three it remained almost unchanged. In the — 90° an increase in frequency was obtained in three animals, in one it was practically unchanged, while one showed a slight decrease. A distinct decrease in amplitude occurred in both positions. Only one determination gave an increase in amplitude, but in three others the same animal showed a decrease. One animal showed a considerable increase in ventilation, which was brought about by a great increase in frequency. This animal will, however, be excluded when judging the results on account of the later occurrence of unusually accelerated breathing indicating some sort of irritation, possibly from aspirated mucus. Apart from this, when carrying out 14 determinations on 5 animals, ventilation in 11 showed a distinct decrease, while it increased somewhat in one determination, and in two determinations on one animal remained practically unchanged.

A typical example is given below (rabbit, 2.5 kg).

The sinus regions intact	Breaths pr min.	Average breaths in ml.	Ventilation ml/min.	Ventilation in the new position in % of the ventilation in the horizontal position
Horizontal position <sup>1</sup> — 45° position <sup>2</sup>	40.5	18.18	736	94.4 %
	40.4	17.20	695	
Horizontal position <sup>1</sup> — 90° position <sup>2</sup>	41.1	17.34	713	87.1 %
	41.7	14.90	621	
The sinus regions denervated on both sides				
Horizontal position <sup>1</sup> — 45° position <sup>2</sup>	31.3	18.32	573	113.2 %
	35.8	18.12	649	
Horizontal position <sup>1</sup> — 90° position <sup>2</sup>	33.8	19.02	643	77.9 %
	32.1	15.60	501	

<sup>1</sup> Just before changing to next position.

<sup>2</sup> Calculated from the beginning ca 10 seconds after changing to position in question, ca 15 breaths onwards.

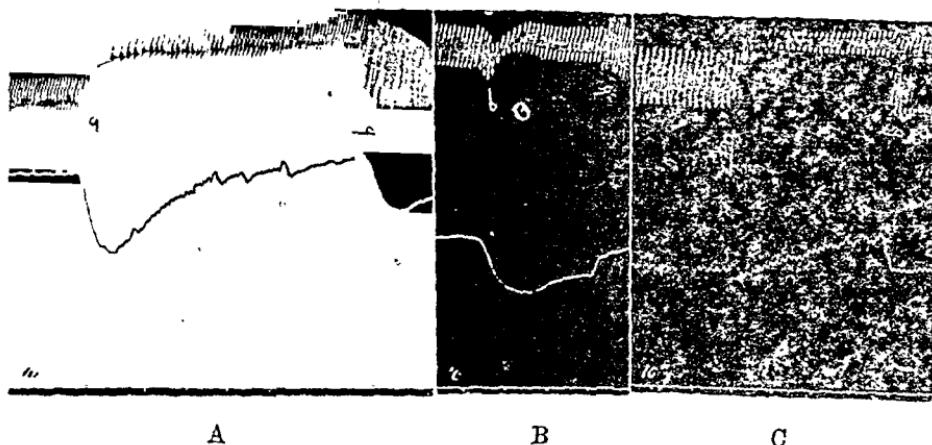


Fig. 1.

- A. Rabbit, 2.3 kg. Change to vertical head-up position. Decrease in frequency and amplitude. Vagi intact.
- B. Rabbit, 2.7 kg. Change to vertical head-down position. Definite decrease in amplitude. Vagi intact.
- C. Rabbit, 2.7 kg. Change to vertical head-up position, after dividing both vagi. Increase in frequency and considerable decrease in amplitude.

After denervation an increase in frequency was obtained throughout at  $-45^\circ$ . At  $-90^\circ$  an increase was obtained in one animal, while another showed a somewhat diminished frequency. A decrease in amplitude was obtained throughout. Experiments were carried out at  $-45^\circ$  on three animals and at  $-90^\circ$  on two. Ventilation was recorded to begin ca 10—20 seconds after the changing of posture. At  $-90^\circ$  there was a decrease in every case, while it increased somewhat at  $-45^\circ$  in three determinations, and in one there was a slight decrease.

It is evident from the above that there was a decrease in amplitude both before and after denervation when changing to the head-down positions, while there was hardly any change or an increase in frequency. There is too little material to determine whether there is any significant difference in these positions before and after denervation. The difference is slight, and in order to obtain accurate results careful consideration would have to be taken to several factors, e. g. the rapidity with which the change of position is carried out, fatigue etc.

After dividing both vagi, too, an equally great decrease in ventilation was produced when changing to  $+45^\circ$  and  $+90^\circ$  as there was before. This decrease was caused by a very pronounced decrease in amplitude, while the frequency somewhat decreased only once in the 7 determinations that were made on the two

animals, apart from this it showed a distinct increase. This increase of frequency is in agreement with the researches of other investigators, who established the fact that the decrease in frequency obtained when changing the position of a rabbit to the vertical head-up disappears after dividing the vagi.

In connection with the head-down positions an increase in frequency and a decrease in amplitude has also been obtained after dividing the vagi. In one animal ventilation showed a slight decrease, while in the other there was a slight increase.

A typical example is given below (rabbit 2.7 kg).

Vagi intact	Breaths pr min.	Average breaths in ml.	Ventilation ml/min.	Ventilation in the new position in % of the ventilation in the horizontal position
Horizontal position <sup>1</sup> + 90° position <sup>2</sup>	57.7 49.2	17.30 10.98	998 540	54.1 %
Horizontal position <sup>1</sup> + 90° position <sup>2</sup>	54.9 52.9	18.84 10.66	1,034 564	54.5 %
Horizontal position <sup>1</sup> + 45° position <sup>2</sup>	56.1 45.8	18.30 14.18	1,027 649	63.2 %
Horizontal position <sup>1</sup> — 45° position <sup>2</sup>	54.1 54.1	21.86 18.56	1,183 1,004	84.9 %
Horizontal position <sup>1</sup> — 45° position <sup>2</sup>	56.1 56.6	19.66 20.66	1,103 1,169	105.9 %
After dividing both vagi				
Horizontal position <sup>1</sup> + 90° position <sup>2</sup>	33.3 39.5	28.66 12.40	954 490	51.4 %
Horizontal position <sup>1</sup> + 90° position <sup>2</sup>	37.2 42.9	28.64 10.70	1,065 459	43.1 %
Horizontal position <sup>1</sup> + 45° position <sup>2</sup>	33.9 37.0	28.46 16.38	965 606	62.8 %
Horizontal position <sup>1</sup> + 45° position <sup>2</sup>	36.6 40.3	29.62 15.68	1,084 632	58.3 %
Horizontal position <sup>1</sup> — 45° position <sup>2</sup>	39.2 40.0	27.86 25.10	1,092 1,004	91.9 %

*Cat.* An experiment has also been carried out on cat. A definite decrease in ventilation was produced when changing to

<sup>1</sup> Just before changing to next position.

<sup>2</sup> Calculated from the beginning ca 10 seconds after changing to position in question, 10 (head-down positions) or 15 (head-up positions) breaths onwards.

+ 45° and + 90° both before and after denervation. The frequency, which is considerably lower than in rabbit, showed a slight tendency to increase or showed hardly any sign of change, while there was a decided decrease in amplitude.

When changing to — 90° both before and after denervation there was a decrease in ventilation caused by decrease in amplitude, while in frequency there was scarcely any change or only a slight increase. At — 45° there was no change of ventilation worth mentioning before denervation, whereas afterwards a slight decrease was obtained.

The decrease in ventilation when changing to the head-up position is probably depending on a fall in the cerebral blood pressure. A fall in blood pressure is in most cases obtained in the carotid arteries when changing to the head-up position (cf. EDHOLM, 1940).

### Summary.

When changing an anaesthetized rabbit from a horizontal to a head-up position, a considerable decrease in ventilation was obtained, this being greater at 90° than at 45°. There was a decrease both in frequency and amplitude. When reverting to the horizontal position, an accelerated respiration above the normal was caused, but the normal state afterwards returned.

When changing the animal to the head-down positions the effect was not so pronounced as in the above mentioned case. The frequency was not largely affected or increased, whereas the amplitude showed a decided decrease. Thus the former often increased the ventilation, while the latter decreased it. Ventilation decreased in general.

After the denervation of the two sinus regions, the same effect was brought about in the head-up positions as has been described for intact animals. There seemed, however, to be a tendency to a relatively greater decrease in the amplitude than in the frequency in comparison with intact animals. There was generally speaking an increase in frequency and a decrease in amplitude in the head-down positions.

After dividing the vagi the decrease in ventilation when changing to the head-up positions was as great as in intact animals. The decrease was brought about by considerable decrease in amplitude. Frequency increased in both experiments. A slight increase in

frequency and decrease in amplitude was obtained with the head-down positions.

A decrease in ventilation was obtained both before and after the denervation in an experiment on cat when changing to the head-up position, and this, too, was usually the case with the head-down. The decrease in amplitude played the decisive part.

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## Influence of Oxygen Inhalation on the Chemo- receptor Activity of the Sinus Region in Rabbits.

By

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Received 10 October 1943.

When oxygen was substituted for air, a moderate decrease of ventilation, arterial blood pressure and pulse rate was observed in anesthetized cats and dogs (EULER and LILJESTRAND 1942). Denervation of both sinuses abolished the effect on respiration, whereas the blood pressure reaction was sometimes undisturbed, but in other cases became reversed, so that oxygen inhalation now caused an increase. This was always found when the sinus denervation was combined with section of both vago-depressor nerves, though this last operation alone had no influence on the blood pressure reaction after oxygen. The slowing of the pulse rate due to the oxygen administration largely disappeared when both vagotomy and sinus denervation had been performed.

It seemed of interest to extend these observations to the rabbit. The animals were anesthetized with 1.4 g urethane per kg body-weight intravenously. Body plethysmograph was employed, and the experiments were performed in the way described for cats and dogs. The rabbits were breathing either spontaneously through Müller valves, or constant artificial respiration was maintained.

When oxygen was breathed instead of air, the typical reduction of respiratory amplitude and rate was observed (fig. 1). After air had been given again, an increase of amplitude and frequency was always obtained. After vagotomy the effect of oxygen inhalation on respiration still persisted, though possibly it was

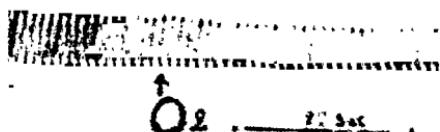


Fig. 1. Rabbit, intact. Respiration curve. At O<sub>2</sub> oxygen is admitted.

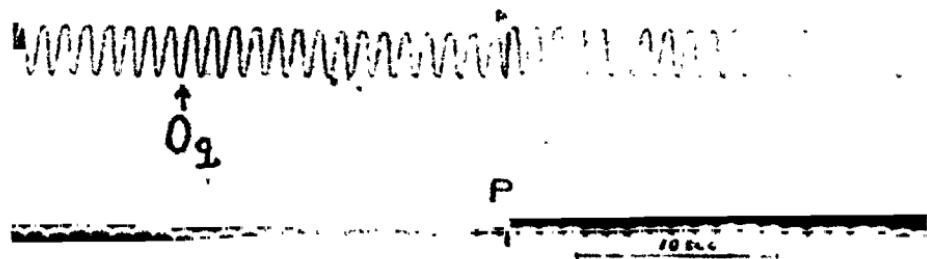


Fig. 2. Rabbit. Vagotomy. Upper curve respiration, lower curve blood pressure. At O<sub>2</sub> oxygen is given. At P a pause of about 2 minutes.

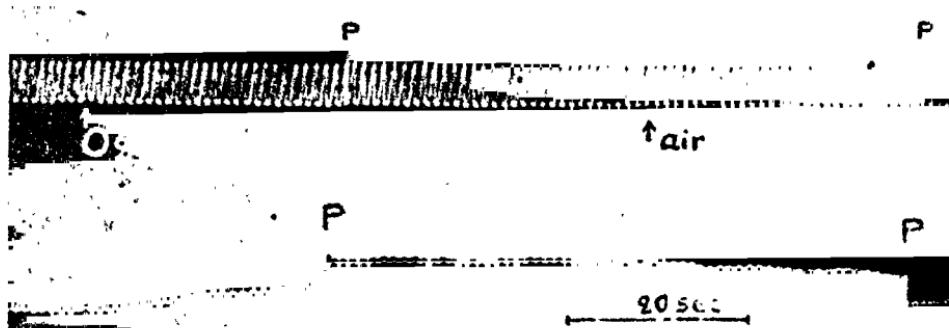


Fig. 3. Rabbit. Both sinuses denervated. Vagodepressors intact. Upper curve respiration, lower curve blood pressure. At O<sub>2</sub> and air oxygen, resp. air is given.  
At P a pause of about 2 minutes.

somewhat reduced (fig. 2); a certain compensation took place during the first few minutes. If both sinuses had been denervated, with or without preceding vagotomy, no reduction of respiration after oxygen was found (fig. 3); sometimes a small increase of ventilation was observed. This is in full conformity with the results obtained on cats and dogs.

With regard to the arterial blood pressure, only small and irregular effects were obtained after oxygen (table 1), and this was also found after section of the vagodepressor nerves. During artificial respiration, a small decrease was usually observed.

When denervation of the sinuses had been performed, however, the blood pressure, with one exception, always rose when oxygen was inhaled, sometimes to a rather considerable extent (table 1, fig. 3). These results are also in complete harmony with those obtained earlier for cats and dogs.

Table 1.

*Percentage Alteration in Blood Pressure and Pulse Rate in Rabbits when Oxygen is inhaled instead of Air.*

s = spontaneous, a = artificial respiration.

Experiment	Normal animal		After vagotomy		After denervation of both sinuses		After vagotomy and denervation of both sinuses	
	Blood pressure	Pulse rate	Blood pressure	Pulse rate	Blood pressure	Pulse rate	Blood pressure	Pulse rate
1. s . . . .	+ 6.6	+ 1.9			+ 19.3	0	+ 12.9	+ 0.7
	- 0.8	- 0.5			+ 19.2	+ 0.7	+ 17.0	+ 3.2
	+ 5.7	- 4.0			+ 20.9	0	+ 12.3	
2. s . . . .	+ 4.0	- 1.0	- 1.5	- 0.4			+ 3.8	- 0.5
	0	0	- 1.0	- 0.8			+ 28.4	- 1.7
	+ 3.0	- 0.3	+ 1.0	- 0.4				
3. a . . . .	+ 1.4	- 0.8	- 3.8	- 1.1			+ 19.0	- 0.6
	- 6.8	- 1.0	- 2.5	- 1.1			+ 17.0	0
	+ 1.5	- 1.0	- 8.9	- 0.5			+ 30.6	+ 1.1
4. a . . . .	- 1.3	- 0.9			- 2.6	+ 0.5		
	- 0.9	- 0.5			+ 9.4	0		
	- 0.8	- 0.2			+ 7.0	- 0.8		

With one single exception, the pulse rate showed a small fall when oxygen was substituted for air in the intact animal, and after vagotomy. No definite effect was observed after denervation of the sinuses.

### Summary.

In rabbits oxygen inhalation seems to have the same influence on the chemoreceptor activity of the sinus region, as had been established earlier for cats and dogs; with the modification, however, that the decrease of the blood pressure in the intact animal was less constant.

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## A Method for Transecting the Pituitary Stalk of the Rat.

By

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Received 3 September 1943.

Transection of the pituitary stalk has given divergent results in different experimental studies of the pituitary body and its functions. For instance, whereas histological alterations in the gonads were found by HARRIS (1937) as well as WESTMAN and JACOBSSON (1937, 1940) after stalk section in rabbits, no such changes could be detected by BROOK (1938). In the rat WESTMAN and JACOBSSON (1937, 1938) as well as HEROLD and EFFEKMAN (1938) found changes in the sexual cycle or morphological changes in the gonads, while UOTILA (1939) and DEMPSEY and UOTILA (1940) state that no alterations of this kind followed stalk section.

Section of the pituitary stalk of the rat can be performed by a subtemporal approach according to GIRAGOSSINTZ (1934), while the parapharyngeal route was used in a method elaborated by RICHTER (1930, 1933). From the descriptions published and from later investigations it is evident that the stalk cannot be seen during the operation, and the number of successful total sections is not large. UOTILA (1939) states that he can make the stalk visible, but does not explain how this is technically possible. However, on photomicrographs published by him (1939) and by DEMPSEY and UOTILA (1940) it is to be seen that cells from the pars tuberalis pass without a break into the anterior lobe. As WESTMAN, JACOBSSON and HILLARP (1943) have shown, however, severance of the connection between the infundibulum and the

posterior lobe as well as between the pars tuberalis and the anterior lobe is of the greatest importance.

*The methods hitherto described for the severance of the pituitary stalk of the rat do not ensure a complete section, sufficient consideration not having been paid to those technical details which are necessary to bring the stalk under ocular control. There would therefore seem to be some motivation for describing a technique developed by me, which has given better results.*

### The Method Employed for the Transection of the Pituitary Stalk of the Rat.

The instruments employed for the operation are illustrated in Fig. 1. The clamp shown in the figure is for screwing firmly around the lower jaw so that the two points project like the tusks of a boar and rest against the table when the animal is laid on its back. The head is also secured by means of a rubber band stretched around the upper incisors. By this means turning movements of the animal's head can be avoided, which is of importance, as it is necessary for the operator to keep the position of the median plane clearly in view. The hooks are made of thin sewing-needles, which are inserted in glass rods. The point of the needle is bent in a gas-flame after it has been pushed into the eye of another needle. The use of needles intended for mounting insects would no doubt yield hooks of still finer gauge. It has been found advantageous to use three such hooks at an operation, one suitable for removing chips of bone, one for opening the dura, and one suited for use on the pituitary stalk. The three spherical drills have a diameter of respectively 2, 1.7 and 1.1 mm. Besides the instruments illustrated a large number of small, hard-rolled cottonwool pellets of different sizes are required.

The operation is performed with the aid of a telescopic loupe (Leitz) magnifying 10 to 12 times and with a focal distance of 14 to 15 centimetres. Good illumination is obtained by the use of a specially constructed lamp, the beam of light from which is directed on the operative area by a concave reflector mounted on the loupe.

The base of the cranium is laid open under ether anaesthesia by the method customary at hypophysectomy. No muscles are sectioned, these as well as the pharynx being pushed aside. Respiration has to take place through a tracheal cannula.

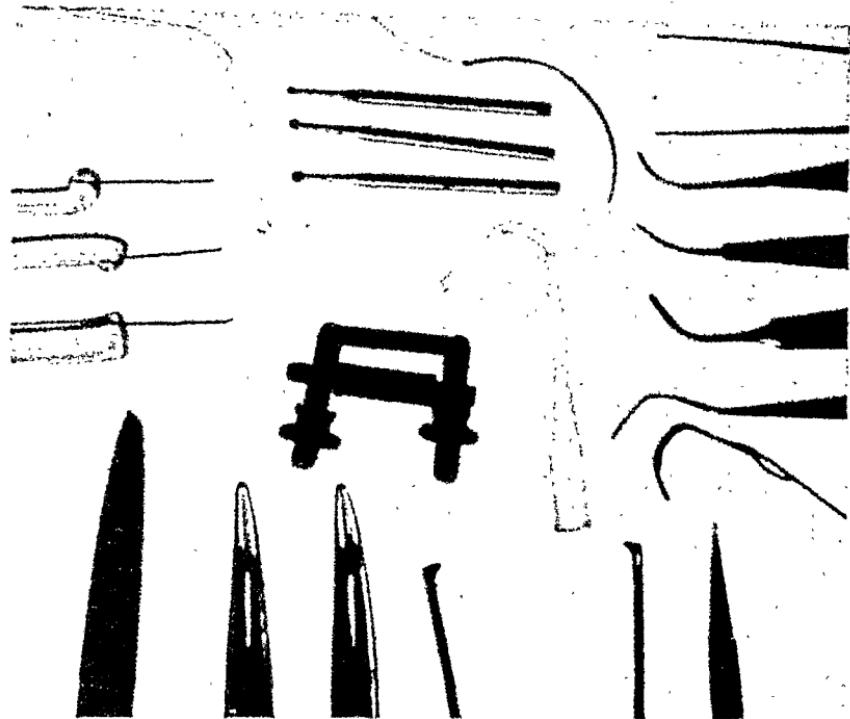


Fig. 1. The instruments employed.

The region orally to the spheno-occipital suture is swabbed clean with a pellet of cottonwool. The pharynx is pushed as far forward as possible, as a rule 4 to 5 mm. orally to the suture, this to avoid perforation of the pars nasalis and pars oralis. Orally, the soft parts are held by a hook with the aid of the left hand, while they are held drawn over to the left by a hook fixed in the operating-table. The centre of the operative field is marked by the median ridge situated behind the suture. The outer lamella of the sphenoid bone is drilled with the coarsest drill in the mid-line just in front of the suture, whereupon the bone marrow is mopped up so as to render the inner lamella visible. A small piece of muscle cut from the pretracheal tissues is inserted in the hole, where it is gently manipulated in between the bony lamellae with pincette and pledget. The hole is then enlarged with the smallest drill by cutting away the outer lamella in the oral part of the hole, alternately drilling and pushing the muscle plug forward under the edge. Sooner or later in the course of the drilling haemorrhage will arise when the transverse sinus is entered, and the arteria canalis pterygoidei may also be injured. A cotton plug placed in the hole readily arrests this bleeding, and after pushing, when

necessary, a little more muscle under the edge of the bone the drilling can proceed. The front edge of the drill-hole should be situated as far anterior to the sphenoid suture as possible. The inner lamella ought no to be drilled until any bleeding that may arise has completely ceased. If the interlamellar plug is not immediately effective, tamponage of the entire hole with cotton and a short interval of waiting usually have the required effect. After the inner lamella has been mopped clean, it is carefully cut away with the two smaller drills. Usually, two or three bone splinters remain, these being removed with one of the small hooks. If any blood then trickles forth, it should be swabbed up cautiously, and a piece of muscle may be placed on the bleeding spot and a cotton tuft over this for a short while. As a rule, no new phase of the operation should be commenced until complete arrest of haemorrhage has been established. The anterior margin of the pituitary body and the stalk are now faintly visible through the dura, which is opened with a thin hook. Should there be any bleeding, which with careful work here is usually very little, a small piece of muscle is temporarily applied to the spot. The stalk, which can now be seen entering the hypophysis, is seized with one of the small hooks and cut off. In doing this care must of course be taken to remove the whole stalk including its borders. At this juncture there is usually an escape of blood and fluid. After the blood has been mopped up, a small piece of muscle is placed between the two bits of stalk and is allowed to remain there. After a short wait to make sure that haemorrhage has ceased the hooks and tracheal cannula are withdrawn. Finally, the organs are restored to their position, after which the operation is concluded with a cutaneous suture.

At a preliminary study of its topographical anatomy the pituitary stalk is most easily recognized by its characteristic vascular delineation with longitudinally straight or sinuously running vessels, which spread out on the hypophysis as over the cone of a funnel after passing along its pipe. The vessels in the pituitary body, on the other hand, appear as a fine network or as arched tops of otherwise concealed loops. The stalk also stands out by its faint yellowish-red tint against the more white base of the brain and by the fact that its lateral edges can be discerned. Fig. 2 is a view of the base of a rat brain, showing the pituitary stalk gripped with a hook.

The results of the operations have been controlled by means of sagittal sections serially cut through the hypophysis and hypothalamus in contiguity. The following procedure has been employed. The bone is removed after being fixed and decalcified, this to render

it easier to cut out the area in question. The remaining piece of bone cannot be lifted straightaway from the dura-enveloped base of the brain, being held there by the trigeminal nerve with its investing fold of dura. The lateral parts of the brain are therefore cut away by sagittal sections until the trigeminal nerve is exposed on one side. After the nerve has been pulled away the piece of bone can be gently lifted from the dura. Since the purpose of the subsequent inspection of the sections is not to ascertain whether the stalk has been obliterated, but the break in continuity mainly appears as scar formations in it, this method would appear to be free from objections.

The fact is that a cicatrization embracing the whole stalk and also the pars tuberalis occupies the first place as a criterion of a satisfactory performance of the stalk-cutting operation. In addition, there should be observable a connective-tissue induration of the posterior lobe, a small connective-tissue scar in the anterior lobe at the point of entry of the stalk, and a break in the continuity of the stalk vessels.



Fig. 3. Operative area, showing complete discontinuity of stalk. 1 pituitary gland, 2 stalk, 3 break in continuity with scar formation at site of stalk transection, 4 posterior lobe (indurated with connective tissue).  $\times 42$ .



Fig. 2. The exposed base of the brain of a killed rat. 1 pituitary gland, 2 pituitary stalk gripped with a hook, 3 optic chiasm, 4 trigeminal nerves.  $\times 10$ .

Since the method described above was devised it has been employed in an experimental series comprising nine rats from 9 to 10 months of age, which were killed two months after the operation. All of the animals survived the operation. In one case the stalk was not brought into view because the trephine hole was made too far caudally, and here a residual stalk-connection was found at the subsequent control of the attempt that was nevertheless made to sever the stalk. In this case

no signs of connective-tissue induration of the posterior lobe were found, whereas in all other cases such signs were present. In two cases the stalk section was partial. Certainly connective-tissue scar was in these cases found with satisfactory localization in the stalk but in a limited area (Fig. 4) parstuberalis cells could be seen to pass over into the anterior lobe. In the remaining six cases complete stalk section had been obtained.



Fig. 4. Partial section of a stalk. 1 pituitary gland, 2 stalk, 3 scar formation, 4 continuous track of cells from the pars tuberalis to the anterior pituitary lobe. The stalk section is not complete. On the figure a vessel is also visible which with the aid of adjacent sections can be shown to extend without a break between stalk and anterior lobe.  $\times 195$ .

*The method set forth here, by enabling severance of the stalk to be carried out under ocular control, ensures a satisfactory result in a larger number of cases than hitherto. At the same time it enables a better evaluation of the result of the operation in the particular case, which enables a better distribution of the animals into experimental groups. Further, the risk of causing unintended lesions is naturally less when the stalk is in view than in a blind operation.*

As an experimental method stalk section in the rat suffers from a certain limitation in its value, since perfectly pure experimental conditions are scarcely obtainable on account of the fact that at the severance of the stalk not only are its nerve tracks broken but also some of its vascular tracks.

### Summary.

Investigations by various authors into the effect of transections of the pituitary stalk upon the action exercised by the pituitary gland on other organs have given varying results. This disagreement, at any rate when the rat has been used as experimental animal, seems to be partially due to the fact that no sufficiently reliable method for transecting the pituitary stalk has been described. In the present paper a method of severing the pituitary stalk of the rat is submitted by which the stalk can be approached by the parapharyngeal route and severed under ocular control. Of nine rats operated on by this method it gave in six cases complete section and in two partial section with pars-tuberalis cells remaining in continuity with the anterior lobe.

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Aus dem Physiologischen Institut der Universität Uppsala.

# Altersveränderungen des Berührungssinnes.

## I. Druckpunktschwellen und Druckpunktfréquenz.

Von

HANS RONGE.

Eingereicht am 17. September 1943.

Eine frühere Untersuchung (RONGE 1942, 1943) hat ergeben, dass die Anzahl der MEISSNERSchen Körperchen in der Fingerbeere mit steigendem Alter stark abnimmt. Ihre Frequenz je Flächeneinheit vermindert sich nämlich von etwa 80 je mm<sup>2</sup> bei Säuglingen auf nur 5 je mm<sup>2</sup> bei alten Leuten. Diese Abnahme fällt zum grössten Teil in die Wachstumsjahre und beruht auf der Flächenvergrösserung der Phalanx beim Wachstum. Die Gesamtzahl der MEISSNERSchen Körperchen in der Fingerbeere sinkt von etwa 6,000 bei Kindern auf 1,000 bei alten Leuten. Diese Involution, welche also ungefähr 80 % der ursprünglich angelegten Nervenendkörperchen umfasst, scheint ziemlich kontinuierlich während des Lebens zu erfolgen und also keine banale, senile Veränderung zu sein (Abb. 1).

Dank v. FREYS (1897) grundlegenden Untersuchungen über die Druck- oder besser Berührungs punkte der Haut wissen wir, dass in den behaarten Hautgebieten, welche ungefähr 95 % der gesamten Körperfläche ausmachen, die Fähigkeit der Berührungsrezeption zum überwiegenden Teil an die Haare gebunden ist. Als rezipierendes Organ betrachtet man den zuerst von BONNET (1878) beschriebenen Nervenkranz um den Haarbalg. In den nicht behaarten Hautgebieten dagegen, d. h. auf den volaren Flächen der Finger, den Handflächen, den Fusssohlen, den Lippen usw., besteht nach v. FREY die morphologische Unterlage der Druckpunkte in den MEISSNERSchen Nervenendkörperchen. Diese sollen auch unter den relativ wenigen, nicht an Haare gebundenen

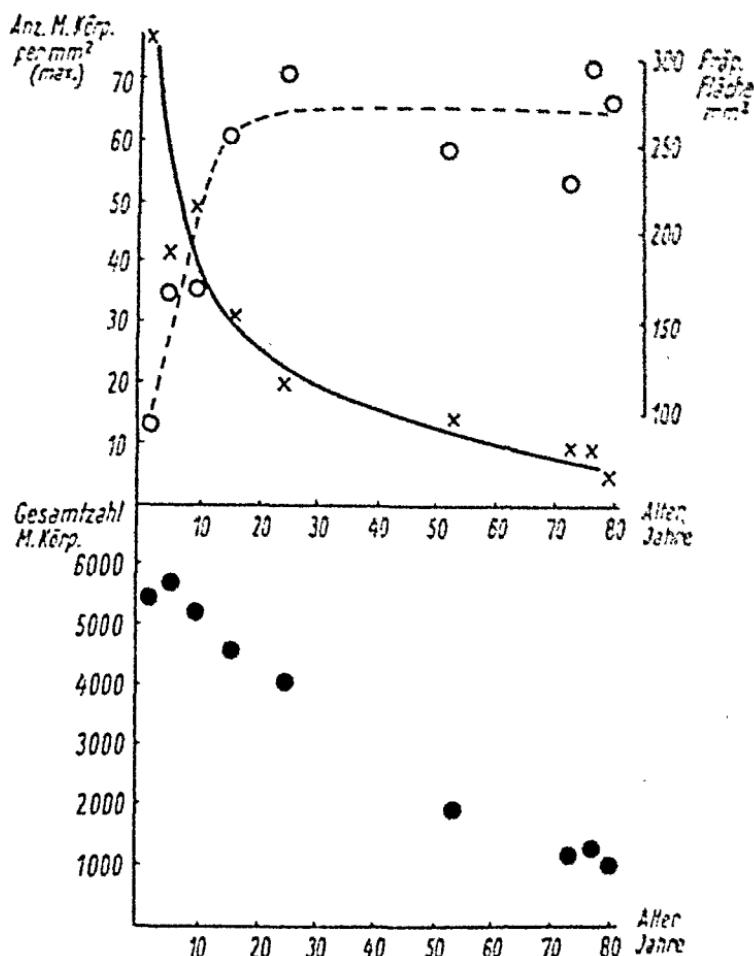


Abb. 1. Im oberen Diagramm markiert die ausgezogene Linie die Anzahl der MEISSNERSchen Körperehen je  $\text{mm}^2$  in der Fingerbeere in verschiedenem Alter. Die gestrichelte Linie gibt die Vergrösserung der Präparatflächen an und somit das Wachstum der Phalanx. Im unteren Diagramm sind die in der Fingerbreite bei verschiedenem Alter gefundenen Gesamtzahlen der MEISSNERSchen Körperehen eingezzeichnet. (RONGE 1943).

Druckpunkten in andern Hautgebieten vorkommen, z. B. auf der volaren Fläche der Unterarme.

Diese Auffassung von der Funktion der MEISSNERSchen Körperehen stützt sich jedoch ausschliesslich auf Wahrscheinlichkeitsgründe. Sie sind die einzigen Nervenendorgane, die in bezug auf Verbreitung und Anzahl den Druckpunkten der betreffenden Hautgebiete entsprechen könnten. Nachstehender Vergleich zwischen den in der Literatur angegebenen Werten für die Anzahl haarfreier Druckpunkte und für die Anzahl MEISSNERScher Körperehen in den entsprechenden Hautgebieten liefert jedoch keinen schlüssigen Beweis für diese Theorie.

Im Thenargebiet beträgt nach v. FREY (1897) die Anzahl Druckpunkte 111—135 und im Hypothenargebiet 119 je  $\text{cm}^2$ . MARTINEZ PERÉZ (1931) gibt die Anzahl MEISSNERScher Körperchen in diesen Gebieten bei Erwachsenen auf 300 bzw. 450 je  $\text{cm}^2$  an. KIESOW und FONTANA (KIESOW 1902) haben 4—7 haarfreie Druckpunkte je  $\text{cm}^2$  auf der Volarseite des Unterarms gezählt, und KRAUSE (RAMSTRÖM 1906) gibt für das ungefähr entsprechende Gebiet die Anzahl MEISSNERScher Körperchen zu 2—3 je  $\text{cm}^2$  an.

BAZETT usw. (1932) haben bei Präputialhaut von beschnittenen Juden nachgewiesen, dass die MEISSNERSchen Körperchen daselbst keine Berührungsrezeptoren sein können. Vielmehr fanden sie unter den Druckpunkten kleine, ungekapselte Nervenendkörperchen. Für die wenigen MEISSNERSchen Körperchen in den untersuchten Hautstücken war keine sichere Funktion festzustellen. BAZETT hält es jedoch für zweifellos, dass die MEISSNERSchen Körperchen in anderen Hautgebieten Druckrezeptoren sind.

D. WATERSTONE (1933) konstatierte, dass die Epidermis und die intraepidermalen Nerven durchschnitten werden können, ohne dass andere Empfindungen als leichte Berührungswahrnehmung hervorgerufen werden. Deshalb verlegt er die Rezeption der Berührungsreize ausschliesslich in die Epidermis und behauptet, dass »andere Funktionen für die MEISSNERSchen und die VATER-PACINISchen Körperchen sowie für andere gleichartige Organe im Corium gesucht werden müssen«.

Gegen die alte Auffassung (v. FREY), dass die intraepidermalen Nervenendigungen Schmerzrezeptoren sind, spricht auch die von v. BAGH (1934) nachgewiesene grosse Schwelleneindringungstiefe für die Hervorrufung von Schmerzempfindungen.

H. WOOLLARD (1935) untersuchte ein ausgeschnittenes Hautstück, auf dem vorher sämtliche Sinnespunkte festgelegt worden waren. Dabei fand er im Gegensatz zu WATERSTONE, dass die schmerzrezipierenden Nervenendigungen in intraepidermalen, dünnen, verzweigten Nervenfasern bestanden. Was die Druckpunkte anlangt, ergab sich, dass ihnen durchgehend Haarbälge entsprachen.

Vor allem durch ZOTTERMANS Untersuchungen (1939) über die elektrophysiologischen Erscheinungen in den sensiblen Hautnerven bei Hautreizung verschiedener Art ist jetzt bekannt, dass an der Fortleitung von Berührungsimpulsen mehrere sowohl morphologisch als physiologisch verschiedene Arten von Nervenfasern teilnehmen, welche mit grosser Wahrscheinlichkeit auch von peripheren Rezeptoren verschiedener Art stammen. Für das Vorhandensein von solchen spricht auch die Beobachtung von BECKER und FRÖHLE (1937), dass bei punktförmiger Betäubung der einzelnen Druckpunkte die dazwischenliegenden Hautgebiete doch nicht jede berührungsrezipierende Fähigkeit verlieren.

Die Ergebnisse der Untersuchung über die Altersveränderungen der Anzahl MEISSNERScher Körperchen schienen mir jedoch eine Untersuchung über eventuelle gleichartige Altersveränderungen der Berührungssensibilität zu motivieren. Diese Untersuchung

umfasste teils Bestimmungen von Druckpunktfrequenz und Druckpunktschwellen in verschiedenen Lebensaltern, worüber hier berichtet wird, teils Studien über die Altersvariationen der Simultanschwellen, welche später veröffentlicht werden sollen.

### Methodik.

Die Untersuchung erfolgte an 10 männlichen Individuen im Alter von 12 bis 76 Jahren. Die Bestimmungen fanden auf der volaren Seite der rechten Handwurzel in einem  $2 \text{ cm}^2$  grossen Hautbereich zwischen den Sehnen des M. palmaris longus und des M. flexor carpi ulnaris statt. Dieses Gebiet wurde unter anderm deshalb gewählt, weil es in der Regel ganz frei von Haaren ist und die an Haare gebundenen Druckpunkte in diesem Zusammenhang nicht interessierten. Die Druckpunktsdichte in diesem Gebiet ist auch von geeigneter Grösse.

Bei der Untersuchung wurde ein Ästhesiometer benutzt. Durch Änderung der Länge des freien Reizhaares wurde ein gesetzmässiges parabolisches Spannungswachstum von  $1.4 \text{ g/mm}$  bis  $6.3 \text{ g/mm}$  erhalten. Das Reizhaar war so abgepasst, dass die empfindlichsten Schmerzpunkte erst bei einem Spannungswert von ungefähr  $5.5 \text{ g/mm}$  reagierten, was bei diesem Ästhesiometer einem Druckwert von etwa  $20 \text{ g/mm}^2$  entsprach. Die gesetzmässige Kontinuität des Spannungswachstums dürfte als ein Beweis für eine hinreichend genaue Reproduzierbarkeit der Reizstärken des Instruments aufgfasst werden können.

Ein Aufsuchen und eine Untersuchung jedes einzelnen Druckpunktes innerhalb des markierten Hautbereichs wäre zu zeitraubend und zu ermüdend für die Versuchsperson gewesen. Deshalb wurde folgende Wahrscheinlichkeitsmethode angewandt.

Mit steigenden Spannungswerten des Ästhesiometers wurde für jeden Spannungswert das Reizhaar an 100 gleichmässig verteilten Punkten des Hautgebietes aufgesetzt. Die Versuchsperson wurde aufgefordert, jede deutliche Empfindung, die sie dabei hatte, durch „Ja“ anzugeben. Die Untersuchungen erfolgten stets unter vier Augen, und alle störenden Momente wurden ausgeschlossen. Die Versuchspersonen wurden so gesetzt, dass sie nicht beobachten konnten, wann das Reizhaar aufgesetzt wurde. Durch Einteilung des markierten Hautbereichs in mehrere kleinere Partien konnten die Reize gleichmässig verteilt werden. Nach jedem Reiz wurde eine Pause von 5—15 Sekunden eingelegt, und nach jeder Reizserie durfte die Versuchsperson 5—15 Minuten ausruhen.

Die wirkliche Anzahl Druckpunkte auf der volaren Seite der Handwurzel eines erwachsenen Mannes wird von Kiesow (1902) zu durchschnittlich  $28.53 \text{ je cm}^2$  angegeben, mit Variationen zwischen 12 und 44 je  $\text{cm}^2$  bei derselben Person. Für den ulnaren Teil desselben Gebiets gibt KIESOW einen Mittelwert von  $22.5$  Druckpunkten je  $\text{cm}^2$  mit Variationen zwischen 12 und 32 an. Innerhalb desselben Gebiets liegt nach KIESOW die obere Grenze für die Schwellenwerte der Druckpunkte bei

2.5 g/mm, während v. FREY (1897) einzelne Druckpunkte mit einem Schwellenwert bis zu 4 g/mm fand. Beide geben die mittlere Schwellenwerte zu 1.25—1.30 g/mm an. Beide Forscher nahmen die Bestimmungen an sich selbst vor. Ihre Ergebnisse sind mit den bei meiner Untersuchung gefundenen Werten sehr gut vereinbar.

## Resultate und Besprechung.

### A. Optimale Reizstärke.

In der nachstehenden Tabelle wird für jede Versuchsperson die Anzahl Berührungsempfindungen bei 100 Reizen der verschiedenen Reizstärken angegeben.

Tabelle.

*Bestimmung der optimalen Reizstärke.*

Alter der Vp. (J.)	% der aufgefassten Reize bei folgender Reizstärke (g/mm)						Ung. optimale Reizstärke (g/mm)
	1.4	2.2	2.7	3.7	4.8	6.3	
12 . . . . .	25	31	40	51	56	96	4.25
12 . . . . .	24	36	43	50	53	80	4.25
22 . . . . .	19	30	37	39	45	73	3.20
23 . . . . .	18	28	35	38	47	72	3.20
28 . . . . .	20	26	29	28	43	64	3.20
43 . . . . .	16	20	21	24	28	54	2.50
62 . . . . .	19	19	27	43	64	—	1.80
70 . . . . .	22	23	32	56	74	—	1.80
72 . . . . .	16	65	73	—	—	—	(1.40)
76 . . . . .	15	55	62	—	—	—	(1.40)

Aus der Tabelle geht hervor, dass mit steigender Reizstärke die Anzahl wahrgenommener Reize anfangs bis zu einem gewissen Grenzwert zunimmt. Bei weiterer Erhöhung der Reizstärke beginnt die Anzahl wieder zu wachsen.

Dies ist folgendermassen zu erklären. Bei den niedrigsten Spannungswerten wird die absolute Schwelle bloss für die empfindlichsten der getroffenen Druckpunkte erreicht. Infolge Erhöhung der Reizstärke reagieren immer mehr Druckpunkte, bis schliesslich bei optimaler Reizstärke sämtliche getroffene Druckpunkte von

der Versuchsperson aufgefasst werden. Bei höheren Spannungswerten werden auch die Schwellen der empfindlichsten Schmerzpunkte überschritten, was die letzte Erhöhung der Anzahl aufgefasster Reize erklärt. Die Versuchspersonen haben dabei auch selbst Schmerzempfindungen angegeben.

*Ein Ausdruck für die totale Anzahl Druckpunkte in dem Gebiet ist die Anzahl der von 100 Reizen aufgefassten (d. h. die Prozentzahl) bei der in dieser Weise bestimmten optimalen Reizstärke.*

### B. Altersveränderungen der Schwellenwerte der Druckpunkte.

In der Tabelle ist die optimale Reizstärke approximativ durch den Mittelwert zwischen den beiden Spannungswerten des Ästhesiometers ausgedrückt, welche die geringste Differenz zwischen den entsprechenden Zahlenwerten aufgefasster Reize ergeben.

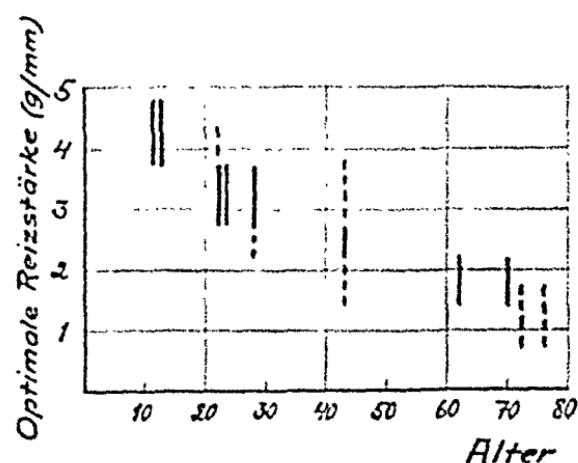


Abb. 2. Reizstärke in verschiedenen Altern.

nämlich von etwa 4.5 g/mm bei Kindern auf 1.5 g/mm bei alten Leuten. Da das verwendete Ästhesiometer keine niedrigeren Spannungswerte als 1.4 g/mm ermöglichte, liefert die Untersuchung keine deutlichen Aufschlüsse über die Veränderungen der absoluten oder der mittleren Schwellen. Die letzteren, ausgedrückt in Schwellenmedianen, scheinen jedoch nach der Tabelle eine gleichartige Senkung mit steigendem Alter zu erfahren.

Die Werte der beiden ältesten Versuchspersonen sind nicht so leicht zu deuten wie die der jüngeren. Die Anzahl aufgefasster Reize steigt hier sehr rasch mit wachsender Reizstärke und gestattet keine sichere Beurteilung der optimalen Reizstärke. Trotz der rasch zunehmenden Anzahl Reize geben die Versuchspersonen

Eine richtigere Darstellung gibt Abb. 2, wo das Grenzgebiet, in dem die optimale Reizstärke liegen dürfte, für jeden Fall angegeben ist. Die gestrichelten Linien bezeichnen Deutungsschwierigkeiten.

Es ist ersichtlich, dass die optimale Reizstärke mit steigendem Alter erheblich sinkt,

auch nicht immer an, dass die überschwelligen Reize mit eigentlichen Schmerzempfindungen verbunden waren, sondern sagten, dass sie dabei ausstrahlende, juckende Empfindungen hätten. Dieselben unterscheiden sich von gewöhnlichen Berührungs-empfindungen auch durch eine dem Untersucher erkennbare längere Latenzzeit zwischen Reiz und Empfindung.

Dies scheint darauf hinzudeuten, dass auch die Schmerzschwellen im Senium niedriger, aber gleichzeitig die Empfindungen seitens der Schmerzrezeptoren schwächer werden. Eine solche »senile Anästhesie« ist eine aus den Kliniken wohlbekannte Erscheinung.

Die Erklärung der altersbedingten Senkung der Spannungswerte der Druckpunktschwellen dürfte in gleichzeitigen Strukturveränderungen der Haut zu suchen sein, die eine relativ grössere Deformierbarkeit der letzteren herbeiführen. Dabei kann abnehmende Dicke der Epidermis und Herabsetzung des allgemeinen Gewebeturgors eine wichtige Rolle spielen.

Bei dieser Deutung steht die altersbedingte Schwellensenkung in gutem Einklang mit der Auffassung von BERNFELDT und FEITELBERG (1932) sowie v. BAGH (1934), dass das adäquate Reizmittel für die Druckpunkte der Grad der Hautdeformation ist. Für kleine Berührungsreize hat nämlich v. BAGH nachgewiesen, dass die absolute Schwelle und andere »topologische« Grössen in erster Linie dadurch bestimmt werden, wie tief der reizerregende Gegenstand in die Haut eindringt. Die mit dem Alter zunehmende Deformierbarkeit der Haut bewirkt also, dass immer weniger Kraft für die wahrscheinlich während des ganzen Lebens ziemlich konstante Schwellendeformation erforderlich ist (Schwelleneindringungstiefe).

Einer solchen Erklärung scheint jedoch die von HÄBLER und POTT (HÄBLER 1939) beschriebene Tatsache zu widersprechen, dass der elastische Widerstand der Haut, gemessen mit SCHADES »Elastometer«, mit steigendem Alter zunimmt, was auf einer gleichzeitigen Sol-Gel-Umwandlung des kolloiden Bindegewebes beruhen soll. Diese Verschiedenheit der Ansichten lässt sich vielleicht dadurch erklären, dass sich HÄBLERS Messungen auf die Deformierbarkeit des Unterhautbindegewebes bezogen, während es sich bei den Hautdeformationen bei Druckpunktstreizen in erster Linie um die Deformierbarkeit der Epidermis handeln dürfte.

### C. Altersveränderungen der Druckpunktfrequenz.

Die bei optimaler Reizstärke gefundene Anzahl Berührungs-empfindungen bei 100 Reizen ist in Abb. 3 wiedergegeben. Wie bei den optimalen Reizstärken ist diese Anzahl durch den Mittelwert zwischen den beiden am wenigsten differenten Werten der betreffenden Serien ausgedrückt.

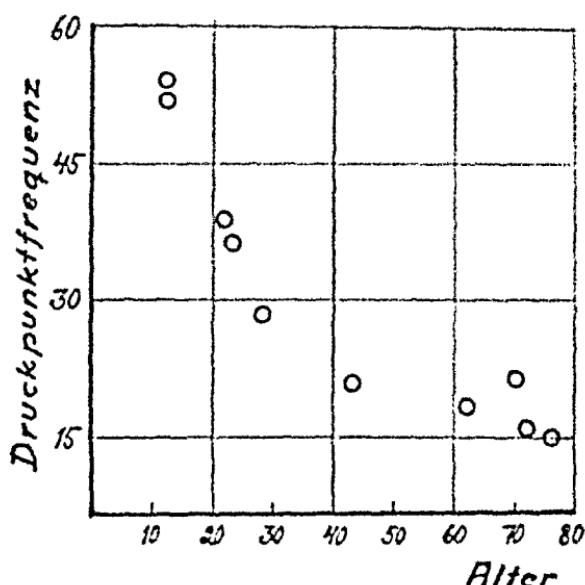


Abb. 3. Druckpunktfrequenz auf der volaren Seite des Handgelenks in verschiedenen Altern.

gende Reduktion, die zwischen den 20er Jahren und dem Senium stattzufinden scheint, kann nur auf einer *Abnahme der absoluten Anzahl Druckpunkte* in dem Hautgebiet beruhen.

Für die Werte der beiden ältesten Versuchspersonen gilt der selbe Vorbehalt, der oben für die optimalen Reizstärken derselben gemacht wurde.

Für eine sichere Beurteilung, ob diese absolute Involution der Druckpunkte kontinuierlich mit steigendem Alter erfolgt, oder ob sie bloss senil bedingt ist, ist das vorliegende Material zu klein. Die gefundenen Werte schliessen jedoch eine kontinuierliche Involution nicht aus.

Infolge der gleichzeitig mit der Involution stattfindenden Verschiebung der Druckpunktschwellen auf niedrigere Werte bleibt die Anzahl der *relativ empfindlichsten Druckpunkte* mit Schwellen bis zu 1.5 g/mm während des Lebens ziemlich konstant (siehe die Tabelle). Dies dürfte die Ursache sein, dass man in der täglichen Praxis nicht so viel von den tatsächlich vorhandenen Altersunterschieden in der Ausrüstung mit Berührungsrezeptoren merkt.

Die augensfällige Abnahme in der Gruppe der 20-jährigen im Vergleich mit den 12-jährigen hängt offenbar in erster Linie mit der während der Wachstumsjahre fortschreitenden Verbreitung der Druckpunkte über eine grössere Fläche zusammen. Aber die etwa 50 % betrags-

Diese altersbedingte Druckpunktinvolution zeigt schlagende Übereinstimmung mit den oben erwähnten Ergebnissen der histologischen Bestimmungen des Vorkommens der MEISSNERSchen Körperchen in verschiedenen Lebensaltern. Diese Übereinstimmung kann jedoch natürlich nicht als ein Beweis für die Theorie betrachtet werden, dass die MEISSNERSchen Körperchen die morphologische Grundlage der Druckpunkte sind. Es ist nämlich sehr wahrscheinlich, dass eine gleichartige Involution, wie sie für die MEISSNERSchen Körperchen nachgewiesen wurde, auch die übrigen Nervenendorgane der Haut betrifft (RONGE 1943).

In den behaarten Hautgebieten, wo die Druckpunkte nachweislich an den Nervenapparat der Haarbälge gebunden sind, dürfte eine gleichartige Druckpunktinvolution als Folge des altersbedingten Haarausfalls vorliegen. Numerische Untersuchungen hierüber scheinen jedoch nicht ausgeführt worden zu sein.

### Zusammenfassung.

Die Altersveränderungen der Anzahl Druckpunkte und der Reizschwellen auf der volaren Seite der Handwurzel wurden studiert. Die Resultate sind folgende:

1. Indem man in Serien von Reizen mit einem Ästhesiometer die Anzahl Empfindungen in einem bestimmten Hautbereich bei steigenden Reizstärken bestimmt, erhält man einen Grenzwert, der *die optimale, d. h. alle Druckpunktschwellen erreichende Reizstärke* angibt (Tab.).

2. Diese optimale Reizstärke nimmt mit steigendem Alter erheblich ab (Abb. 2). Dies erklärt sich wahrscheinlich durch eine mit dem Alter zunehmende Deformierbarkeit der Haut (Epidermis).

3. Die Anzahl Empfindungen bei optimaler Reizstärke ist ein Mass der Druckpunktdichte in dem Hautgebiet. Diese Werte erfahren eine bedeutende Reduktion mit steigendem Alter (Abb. 3). Während der Wachstumsjahre beruht dies in erster Linie auf der fortschreitenden Flächenvergrösserung des Körpers. In der Zeit zwischen dem Abschluss des Wachstums und dem Senium, wo die Druckpunktinvolution nach wie vor deutlich bemerkbar ist, muss die Ursache in einer fortschreitenden Involution der berührungsrezipierenden Nervenendigungen der Haut zu suchen sein.

4. Diese Druckpunktinvolution stimmt gut mit der von dem Verfasser früher beschriebenen Altersinvolution der MEISSNER-

schen Nervenendkörperchen in der Fingerhaut überein. Dies kann jedoch nicht als Beweis für eine berührungsrezipierende Funktion der MEISSNERSchen Körperchen aufgefasst werden.

5. Die Untersuchungsresultate schliessen nicht aus, dass die Altersinvolution der Druckpunkte ziemlich kontinuierlich während des ganzen Lebens weitergeht und also keine banale, senile Veränderung ist.

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## On the Regulation of Circulation during Muscular Work.

By

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Received 25 September 1943.

In an earlier investigation (ASMUSSEN, CHRISTENSEN and NIELSEN 1940) it was shown that the rate of the circulation during muscular work is closely correlated to the intensity of work even when the circulation to the working muscles is blocked, so that it need not be related to the oxygen consumption of the working muscles and hence to the degree of the peripheral vasodilatation. It was therefore put forward as a preliminary assumption that the cardiac output in the steady state of work is regulated by means of cortical impulses in the same way as KROGH and LINDHARD (1913) explained the sudden changes in circulation occurring at the onset of work. In order to study this question we have determined the cardiac output, the pulse rate and the blood pressure in a series of experiments with electrically induced work, the results of which were compared with the values obtained from experiments with normal voluntary work.

### Methods and Results.

The work machine was an angular lever moving on a horizontal axis. One arm of the lever was connected with a spring, the other had pedals to which the feet of the subject could be fixed. The subject was sitting on the floor with half bent knees and his back against the wall. The work consisted in a rhythmic stretching of the legs at a frequency of about 30 per minute. The electrical stimulation was produced by the "Myotensor" and the different electrodes were placed dorsally on the thighs and ventrally on the calves of the subject. The

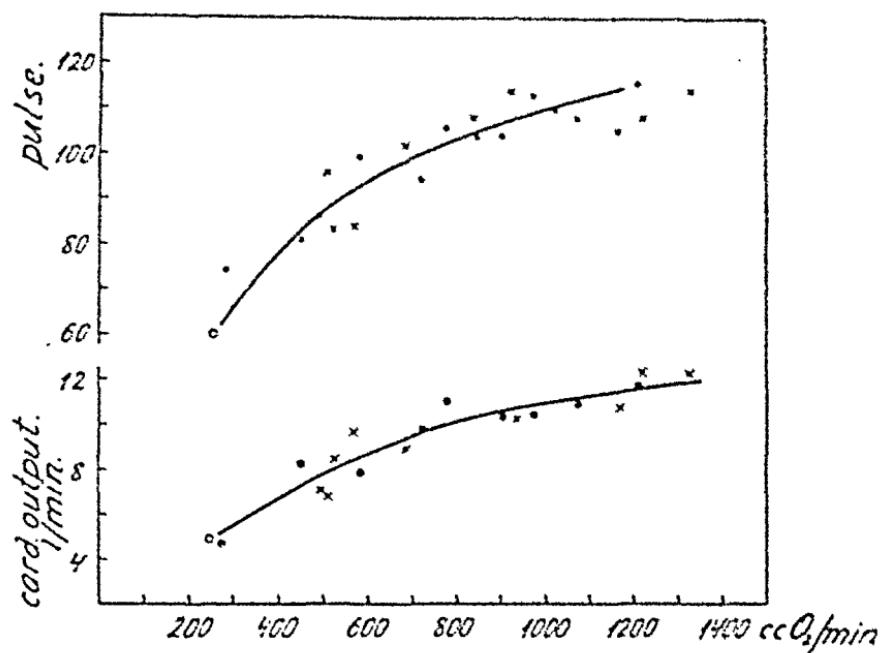


Fig. 1. Cardiac output and pulse rate in relation to oxygen intake. Subject E. A.

- Electrically induced work
- × Voluntary work
- Average of rest values.

indifferent electrode was placed on the back of the subject. The stimulation by the "Myotensor" caused no sensations whatever from the area of skin covered by the electrodes. A more detailed description of the work machine and of the mode of action of the "Myotensor" is presented in an earlier paper (ASMUSSEN, NIELSEN and WIETH-PEDERSEN, 1943).

The oxygen consumption was determined about 15 minutes after the start of the work by the DOUGLAS-bag method and the arterio-venous oxygen difference immediately afterwards by means of the acetylene-method of GROLLMAN.

The main experiments were performed on subject E. A. (35 years, 172 cm., 70 kgm.).

In fig. 1 the cardiac outputs and the pulse rates from the experiments with normal voluntary work and electrically induced work are presented in relation to the oxygen consumption. The work performed involved oxygen consumptions up to about 1,200 cc pr. minute, i. e. about 5 times the resting value. The cardiac output was thereby increased to more than twice the resting value. Correspondingly the pulse rate increased from about 60 during rest to about 120 at the heaviest work performed. The pulse curve of this subject shows for the small work inten-

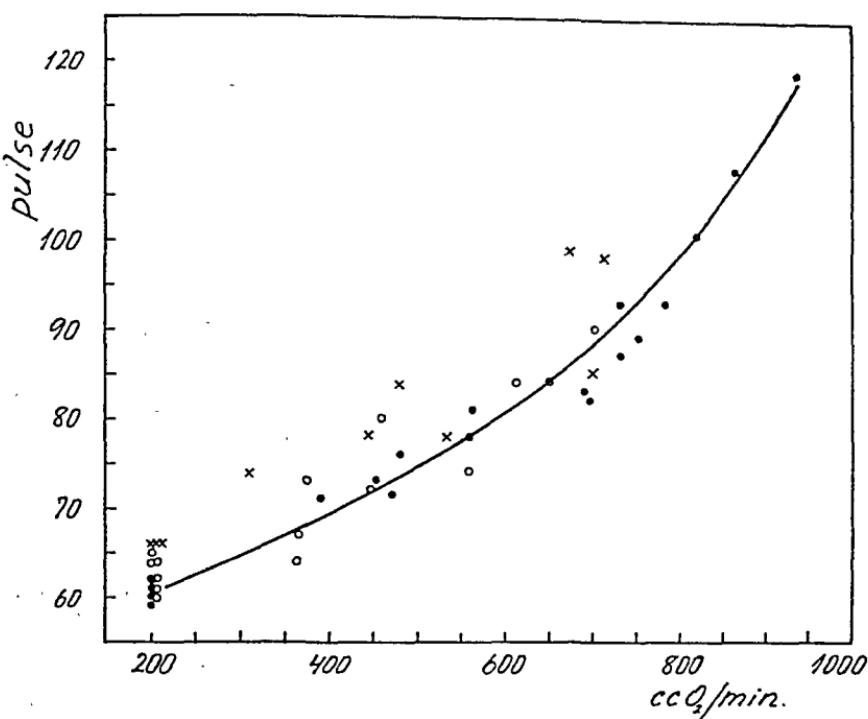


Fig. 2. Pulse rate in relation to oxygen intake. Subject H. Th.

- Electrically induced work
- × Voluntary work on work machine described in text
- Voluntary work on bicycle ergometer.

sities a relatively greater increase than ordinarily. As the figure reveals the cardiac output and the pulse rate increase in the same way corresponding to the oxygen consumption during the two kinds of work.

The arterial blood pressure increased from about 105 mm Hg. during rest up to about 130 mm Hg. during the heaviest work both in the normal voluntary- and in the electrically induced work.

Some additional experiments were made on a subject, H. Th. (57 years old), who suffered from tabes dorsalis, and in whom the ordinary kinesthetic sensations in the lower extremities were completely extinguished. In the electrically induced work this subject could reach an oxygen intake of 700 cc. per minute, and during voluntary work on a specially built bicycle ergometer a steady state at an oxygen consumption of 950 cc. per minute could be obtained. The pulse rate (Fig. 2) and the arterial blood pressure also in this subject increased corresponding to the oxygen intake independently of the kind of work performed.

### Discussion.

It is often assumed (REIN 1935 a. o.) that the increase in the cardiac output during muscular work is caused by pressosensible reflexes as a link in the regulation of the arterial blood pressure. The vasodilatation in the working muscles produces a tendency to a fall in the arterial blood pressure which is supposed — via the presso-sensible zones — to call forth the pulse acceleration, the compensatory vaso-constrictions and the emptying of blood depôts. This theory, however, meets with certain difficulties. It is not easy to understand how the reflex reactions can be brought about at the onset of work, as normally no initial fall in the arterial blood pressure is observed. It is further hard to see how the circulatory changes (increased pulse rate and change in vaso-motor tone) can be maintained in the later stages of work where the arterial blood pressure shows no tendency to diminish but on the contrary is increased.

According to the theory here discussed the rate of circulation during muscular work should be determined by the extent of vaso-dilatation in the working muscles. Work experiments (ASMUSSEN, CHRISTENSEN and NIELSEN, 1940) in which about 50 pCt. of the working muscles were excluded, by means of pneumatic cuffs, from the circulation in the steady state of the work, showed, however, that the cardiac output was not diminished although the oxygen intake of the muscles was approximately halved. Further it was shown that if work was commenced while about 50 pCt. of the working muscles were "cut off" from the circulation, the cardiac output nevertheless rose to the level corresponding to the intensity of the work. These experiments show that the output of the heart in exercise is closely correlated with the intensity of work, but that it need not be related to the extent of the vaso-dilatation.

The same experiments also exclude the possibility that substances liberated in the working muscles via the bloodstream should play any important rôle for the increase in the circulation.

Whereas the results of the above mentioned experiments hardly can be explained by the assumption that the increase in the cardiac output during work is due to the automatic blood pressure regulation, the close relation between intensity of work and rate of circulation suggests, that the increase in the cardiac output during muscular work may be effected; either through

irradiation of the cortical motor impulses or reflexly through sensory impulses arising in the working muscles. The strength of the nervous impulses involved in either case will be closely correlated to the intensity of work.

The experiments here presented, in which the voluntary cortical innervation of the muscles was substituted by the apparatus for electrical stimulation show, that the regulatory mechanism of the circulation was still acting in the normal way. From these experiments it can be concluded, that the regulation of the circulation during muscular work is not brought about by cortical impulses. It therefore seems justifiable to assume, that the increased cardiac output in the steady state of muscular work is brought about reflexly through sensory impulses from the working muscles.

The experiments with the patient suffering from tabes dorsalis showed that this patient could reach a steady state of work corresponding to an oxygen consumption of 4 to 5 times the resting metabolism. This seems — in connection with the normal increase in blood pressure and pulse rate — to indicate, that his circulation has been increased corresponding to the intensity of work. The reflex impulses governing the rate of circulation in exercise therefore seem to be conveyed by sensory nerves outside those nervous tracts, which are known to be destroyed in tabes dorsalis.

Whether the reflex impulses act exclusively upon a circulatory centre or whether an influence of the adrenals may also be involved, can not be decided from these experiments.

In an earlier paper (ASMUSSEN, NIELSEN and WIRTH-PEDERSEN, 1943) it has been shown that also the increase in the pulmonary ventilation during muscular work most probably is brought about by reflex impulses from the working muscles. This together with the results of the present experiments indicates a connection between the regulations of the circulation and the respiration, respectively, in so far as the increase in the cardiac output and the pulmonary ventilation during muscular work both seem to be governed by reflex impulses from the working muscles.

### Summary.

In a series of experiments the cardiac output, the blood pressure and the pulse rate have been determined during electrically induced — and normal voluntary work. The results showed that the

circulation was increased corresponding to the oxygen consumption independently of the kind of work performed. From these results it is concluded, that the cortical impulses play no part in the regulation of the circulation in the steady state of muscular work. This together with other evidence discussed in this paper makes it probable, that the regulation of the circulation during muscular work is governed by reflex impulses from the working muscles.

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## Stretch Potentials of Skeletal Muscle.

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Received 2 October 1943.

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The stretching of an isolated striated muscle in the frog is accompanied by increased heat production and oxygen consumption even if the muscle is rendered inexcitable to electric stimulation by potassium treatment (FENG, 1932). The effect of stretch was further studied in Hill's laboratory by EULER (1935), who was able to confirm this peculiar phenomenon and also found that the effect was reduced after treatment of the muscles with acids and certain surface active compounds, and increased after treatment with alkali or cocaine. As the mechanism of the stretch effects is still obscure, it appeared to be worth while to enquire into the accompanying electrical phenomena.

EINTHOVEN and RADEMAKER (1916) and EINTHOVEN (1918) have recorded variations in the demarcation current as a result of stretching the isolated muscle of a frog. The current variations observed were attributed by the above-mentioned authors to a change in the shape of the muscle when stretched, and a disturbance in the distribution of current-delivering elements.

Against this conception it might be objected that similar variations of current in the measuring circuit, i. e. muscle, galvanometer, and compensating apparatus connected in series, will readily be obtained when an uninjured muscle is stretched. Since the recording apparatus of EINTHOVEN is a current measurer, variations in the resistance of the muscular tissue between the leading-off points would be recorded, in addition to possible true voltage changes.

As it might be anticipated that modern recording technique would yield different results, the following experiments were undertaken in order to study the electrical phenomena recorded from the muscle surface, when the muscle is stretched, with a view to finding out to what extent these are affected by different conditions.

### Methods.

Experiments were made on the gastrocnemius and sartorius muscles of the frog, which were excised with a portion of the bone to which they are attached. The bony part of the preparation was fixed in a muscle chamber by a pin, and the achillis tendon was connected by a thread to an optical spring myograph. The muscle could be stretched by loading the thread. Usually a weight of 25 g was applied. Similar experiments were made also on the tibialis anterior muscle, *in situ*, of the rabbit under urethane.

Potential differences were led off from the muscle surface near the femoral attachment and from the tendon by means of cotton strips soaked in Ringer's solution and attached to chlorinated silver electrodes. In this way displacement of the electrodes to the leading-off points was avoided. The potentials were led to a 4-stage, directly coupled amplifier, which operated a torsion band oscillograph. The muscular movements and the electrical changes were recorded on photographic paper, as described previously by EULER and SWANK (1940).

If, as is usually done, the muscle is connected in parallel with the grid resistance in the input circuit of the first stage, the varying resistance between the leading-off points will affect the grid current more or less as the muscle is stretched, and a fluctuation in the plate current of this valve will be the result. Thus variations in resistance between the leading-off points of the muscle will appear as fluctuations of voltage. Hence, the electrodes were connected with the grids of a push-pull coupled first stage, which made it possible to record true potential variations only.

The demarcation potentials of the resting muscle were measured by means of an electrostatic vacuum tube voltmeter with practically no current ( $10^{-11}$  mA) flowing in the measuring circuit.

Between the recordings the excised frog muscle was soaked in various solutions. Thus the tissue was always kept moist, though suspended in air during the recordings. The ability of the muscle to respond to direct electric stimulation was always controlled immediately afterwards.

Such stimuli as would cause contraction were avoided.

Potentials were led off 1) from intact muscle surface, and 2) from muscles with one leading-off point (tendon end) injured by means of hot Ringer's solution (50–60° C) or by 40% lactic acid.

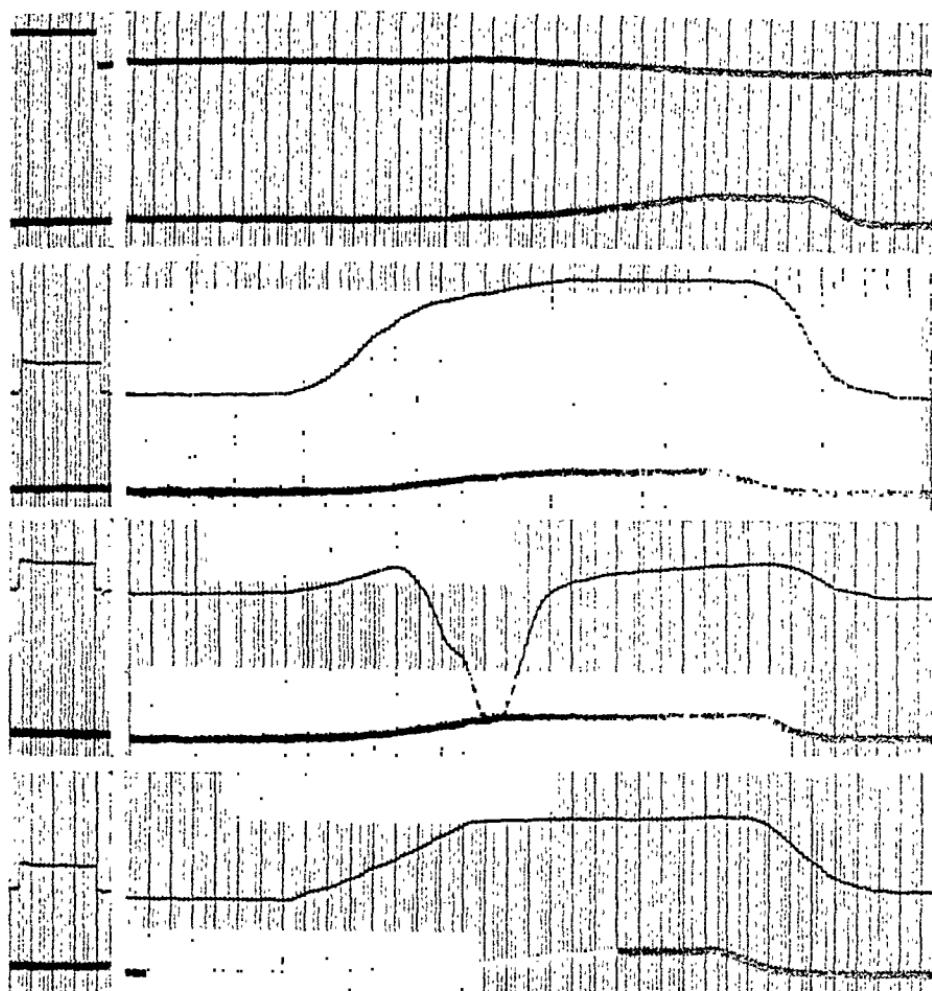


Fig. 1. Frog's gastrocnemius. Upper curve: electrogram, lower curve: mechanogram. Time marks: 1/50 and 1/10 sec.

a) Intact muscle.

b) Tendon end injured by 40% lactic acid.

c) After soaking in hypotonic Ringer's solution (1:20) 15 min.

d) After soaking in isotonic Ringer's solution 30 min.

Load 25 g. Calibration 1 mV.

### Results.

a) *Intact muscle* (frog's gastrocnemius and sartorius, rabbit's tibialis anterior):

Even when the muscle was excessively stretched, only minor deviations from the base-line, probably representing either slight changes of a small demarcation potential or artifacts remaining from changes in resistance between the leading-off points were recorded (Fig. 1 a).

b) *Muscle injured at tendon end* (the same muscles as tested above):

When the muscle was moderately stretched, a change in demarcation potentials was always observed, the muscle belly turning negative in relation to the tendon, showing that a certain amount of depolarization takes place. A typical variation of this kind (stretch potential) is shown in Fig. 1 b. The potential difference decreases to a minimum as the stretching reaches its maximum, and then regains its initial value as the muscle is unloaded.

The extent to which stretch potentials, obtained as mentioned above, are affected by various conditions, was investigated in the following experiments.

c) *Stretching the muscle by varying loads.* With increasing load the stretch potential also increased, proceeding asymptotically to a maximum. In our experiments the stretch potentials did not exceed 10 millivolts, whatever the load or type of muscle. The maximum value varied from species to species (maximal difference 5 mV), but it never compensated the demarcation potential to zero. Apart from the more or less lengthened plateau of the curve, its shape bore no relation to the duration of the stretching. As the muscle was unloaded the demarcation potential always regained its original value simultaneously.

d) *Denervated and curarized muscles.* By division of the sciatic nerve 7 to 12 days before the experiment (frog's gastrocnemius, rabbit's tibialis anterior), the muscle was deprived of its denervation as tested by indirect stimulation. No change of the stretch potential was observed in the muscles examined. Complete curarization (frog's gastrocnemius) yielded the same results.

After it had been established that stretching consistently produced a decrease in the demarcation potential, we studied the effect of modifications in the ionic milieu and of certain drugs on this effect, in order further to elucidate the nature of the potential change.

In the following experiments, muscle preparations of frog's gastrocnemius were used throughout. The soaking solution was changed in respect of the content of various cations and anions and allowed to act for  $\frac{3}{2}$ —2 hours. Further, the effect of adding pharmacologically active agents to the soaking Ringer's solution was studied.

e) *Potassium.* Isotonic solutions of KCl and KNO<sub>3</sub> always diminished the height of the plateau (Fig. 2 b). After an hour's washing in Ringer's solution, however, a striking change in the electrical picture became evident. Thus the stretch was accompanied by a sharp initial increase in potential difference (fall in curve), which then gradually returned to a level just below the equipotential line (Fig. 2 c). The normal shape (Fig. 2 a) did not return after additional soaking in Ringer's solution for 2 hours. On the other hand, renewed treatment with a solution of potassium chloride resulted in the reappearance of the original shape (Fig. 2 b).

f) *Sodium.* Isotonic NaCl solution was without any detectable effect.

g) *Calcium.* Isotonic CaCl<sub>2</sub> solution caused a decrease in the height of the plateau of the electrogram, which became normal again after the muscle had been washed in Ringer's solution.

h) *Hypotonic solution.* After the muscle had been treated with various degrees of hypotonic

Ringer's solutions in decreasing concentrations, a change in the stretch potential constantly occurred at a concentration of about 1:20. During the stretch the electrogram showed an initial slight decrease in potential (rise in curve deflection) between the leading-off points. This decrease then suddenly

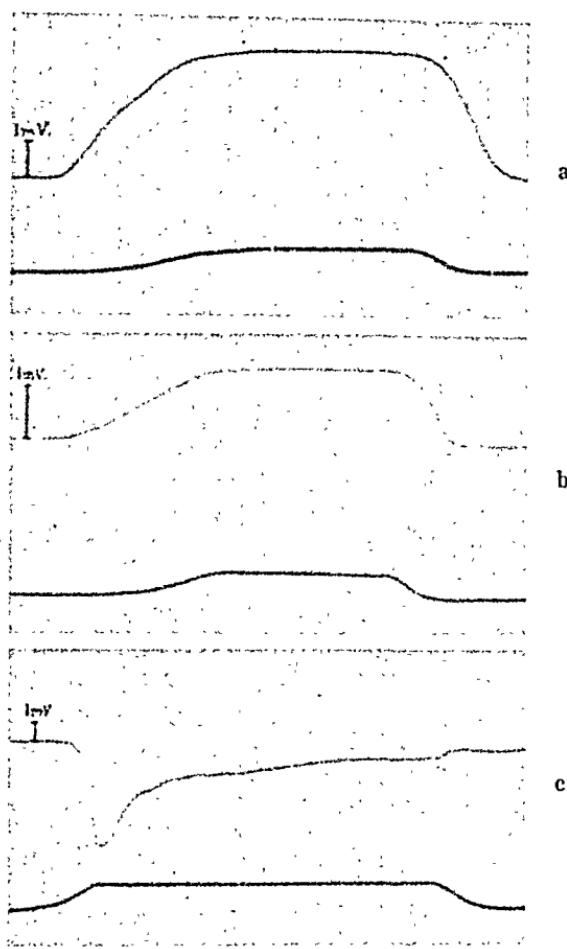


Fig. 2. Frog's gastrocnemius. Upper curve: electrogram, lower curve: mechanogram. Time marks: 1/50 and 1/10 sec.

a) Normal stretch electrogram.

b) After soaking in isotonic KCl 30 min.

c) After washing in Ringer's solution 30 min.

Load 25 g. Calibration 1 mV.

changed into an increased potential difference, which rather quickly returned to a level above the equipotential line, and finally, the muscle being unloaded, reached the original demarcation value (Fig. 1 c). In this case, however, the normal shape reappeared after washing in isotonic Ringer's solution (Fig. 1 d).

i) *Novocaine*. When the muscle was immersed in 1—2 % solution of novocaine hydrochloride for half an hour, the stretch potential gradually subsided and finally disappeared. After an additional hour's soaking in Ringer's solution the muscle had regained its normal stretch potential.

k) *Adrenaline* (1 : 100 000) and *atropine* (1 : 1 000) did not cause any detectable change in the stretch potential, even after one hour's action.

In all the experiments the changes in the mechanograms and electrograms always started from and returned to their base-lines simultaneously.

### Discussion.

For mapping out the distribution of potential on the surface of a muscle the ideal method is obviously one in which changes in potential can be measured relative to some unvarying standard. A method of this kind, introduced by CRAIB (1928), has presented considerable advantages in many respects, as compared with the more common one of recording differences in potential between two points on the muscle surface. As has been recently pointed out by BISHOP and GILSON (1937), however, the potential of the standard electrode does not actually remain constant under all circumstances, e. g. in the case of recording action potentials. The surrounding medium, in which the standard electrode is fixed, changes its average level potential as a result of ion mobilization from the inside to the surface of the muscle. This objection cannot, however, be advanced in the case of a resting muscle, injured or not, but of course in the case of a muscle being stretched.

Hence, in the present experiments, the old method of comparing the potentials of two points on the muscle surface was employed. It might here be worth mentioning that, as has been clearly shown by SAMOJLOFF (1899), CRAIB (1928), KERBER (1933), and SUGI (1940), the amount of current flowing per unit of time

in the measuring circuit is modified by the quality and quantity of the medium surrounding the muscle. Though in this way the actual potentials may be changed by the environment of the muscle, the sites of origin of positive and negative ions are certainly not influenced thereby. In the present case, the muscle was suspended in air, but in practice its environment consisted of a thin layer of conducting medium. Owing to the change in the surrounding electrical field imposed by the stretching, the use of a standard electrode, as in the case of a contracting muscle, appears to be less favourable for recording the ensuing potential change. For this reason we placed both electrodes on the surface of the muscle.

In all the experiments on injured muscle soaked in isotonic Ringer's solution, the leading-off point of the muscle belly turned relatively negative as the muscle was stretched, i. e. the demarcation potential diminished. This must indicate a decrease either of the positivity beneath the belly electrode, or the negativity beneath the tendon end electrode, or both. The first alternative is the most acceptable, as it can be explained on a purely mechanical basis. When the muscle is stretched, the demarcation region will move in the same direction as the pulling force, because of the relative rigidity of the injured tendon end as compared to the elastic muscle belly. The result will be an increased distance between the demarcation region and the point of the muscle surface beneath the belly electrode, just as if this electrode had been moved in a direction away from the demarcation region. This anticipation of ours was confirmed by experiments with a wandering electrode on a resting, injured muscle. The result was a decrease in the demarcation potential as the distance between the electrodes was increased, as could be expected in accordance with the results of SUGI (1940), who has made very comprehensive measurements of the distribution of potential on the surface of resting, injured muscles. He observed that the positivity and negativity of the uninjured and injured surfaces of the muscle respectively decreased with increasing distance from maximum points near the demarcation region.

In view of the peculiar change in shape of the normal stretch electrogram in our experiments on the effect of various electrolytes and pharmacological agents, where a temporary increase in the demarcation potential (polarization) took place, one must assume a redistribution of charges in the uninjured muscular

surface during the stretching. The electrograms obtained in these cases might be regarded as difference or summation curves, in which one component is the normal stretch potential, and the other represents the ion mobilization.

The depressive action of potassium and calcium salts and of novocaine strongly indicates the presence of depolarization phenomena in the uninjured muscular surface as the muscle is being stretched. Again, after treatment with potassium salts and subsequent washing, or after hypotonic Ringer's solution, a relative polarization appears during the stretch, tending to restore the original demarcation potential.

Studying the rates of heat production of the sartorius muscle of *Rana temporaria* after stretching the muscle under various conditions, EULER (1932) found an increase after treating the muscle with cocaine hydrochloride. The relation of this effect to possible alterations in the permeability of the muscular surface or interfaces was implied. It is of interest that novocaine, probably for the same reasons, causes alterations in shape of the stretch electrogram.

### Summary.

True stretch potentials from the surface of striated injured muscle (frog's gastrocnemius and sartorius, rabbit's tibialis anterior) have been recorded.

The cause of the normal stretch potential is partly ascribed to a lengthening of the distance between the source of demarcation E. M. F. and the leading-off point on the uninjured surface of the muscle during the stretching.

Denervation or treatment with curare did not affect the stretch potential.

Potassium and calcium salts, as well as novocaine, were found to change the shape of the normal stretch electrogram. It is suggested that these changes are caused by depolarization phenomena in the surface of the uninjured part of the muscle.

Soaking the muscle in hypotonic Ringer's solution or washing the muscle after potassium treatment caused a reversal in the stretch potential (relative polarization).

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# The Mechanism of Renal Excretion of Fructose and Galactose in Rabbit, Cat, Dog and Man

(with Special Reference to the Phosphorylation Theory).

By

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Received 2 October 1943.

## Introduction.

Great interest has been taken in the renal excretion mechanism of various sugars, especially with regard to the filtration-reabsorption theory of the kidney function. Various sugars — pentoses, hexoses and polysaccharides — to some extent have been classified according to their active reabsorption in the kidney tubules. Glucose for instance has been proved to be a typical threshold substance. On the other hand, inulin is filtered through the glomerular membrane, but is not at all absorbed or excreted by the tubules; inulin is now considered to be the substance best qualified for the determination of glomerular filtration. Other non-metabolized sugars (xylose, sucrose and raffinose) in contradistinction to inulin are partly reabsorbed by the kidney tubules (RICHARDS, WESTFALL and BUTT (1934) and other investigators).

Little is known about the renal excretion mechanism of fructose and galactose, although galactosuria and fructosuria are well known clinical findings. Fructose and galactose are selectively absorbed by the intestinal mucosa; the fructoshaemia following the administration of fructose orally is relatively moderate, as this hexose is absorbed slowly by the intestinal mucosa, but assimilated very intensely by most tissues, especially muscular

and liver tissues. On the contrary, galactose, when administered orally, will cause a relatively considerable galactosaemia, as a result of its rapid intestinal absorption and its low rate of assimilation mainly in the liver and intestinal mucosa.

The mechanism of the active tubular reabsorption of hexose is not yet known. The best known theory of active hexose absorption in intestinal mucosa and kidney tubules is the hypothesis advanced by LUNDSGAARD and VERZAR's collaborators, LASZT and WILLEBRANDT in 1933. According to this hypothesis the active absorption of hexose depends upon an intermediate phosphorylation of the hexose during its passage through the cells of the kidney tubules and intestinal mucosa respectively. The demonstration of an intense phosphorylation of fructose and galactose in the intestinal mucosa of living animals argued strongly in favour of the phosphorylation theory (KJERULF-JENSEN, 1942). Phosphorylation of hexoses *in vitro* in kidney tubular tissue was demonstrated by KALCKAR (1938). The well known fact that phlorizin accumulated in kidney tubules is capable of blocking the active glucose reabsorption favours the assumption of a phosphorylation of hexoses in kidney tissue *in vivo*, for phlorizin may be considered to be a specific inhibitor of the function of the adenylic acid system, which is probably the main phosphate transporting mechanism of the tissues.

CORI (1925) and later investigators have demonstrated the different rates of intestinal absorption of various monosaccharides; in rats the absorption rates found by CORI, given in percentage of the glucose absorption rate, were: galactose 110, fructose 43.

The present investigation was undertaken for the purpose of examining the excretion of fructose and galactose through the kidneys. In particular, the reabsorption of these two hexoses in the tubules was compared with reabsorption of glucose. As to the mechanism of this reabsorption (possibly a phosphorylation), investigation should clear up several problems involved in the absorption of the carbohydrates. If it can be proved that the mechanism of reabsorption for fructose and galactose is susceptible to poisoning with phlorizin, it would argue that an intermediate phosphorylation explains the passage of the two hexoses through the cells of the tubules. It would also be additional evidence for the share of this process in the reabsorption of the carbohydrates if it could be proved that the various hexoses compete

for the factors necessary to phosphorylation, such as the energetic oxydo-reductive systems and the adenylic-acid system as phosphate donors.

### Survey of Experiments.

1. The demonstration of fructose and galactose as threshold substances.
2. The relation of fructose and galactose reabsorption to the quantities filtered.
3. The dependence of the reabsorptive mechanism of fructose and galactose on:
  - a) the variation in the output of urine.
  - b) poisoning with phlorizin.
  - c) the saturation of the hexose-reabsorptive mechanism with glucose.

### Methods.

In the experiments on rabbits, cats and dogs the animals were narcotized with chloralose, except in some few experiments on rabbits and dogs. The fructose and galactose were administered in concentrated solutions, intravenously, at a constant rate during the experimental period.

Blood samples were taken from the carotid arteries. The urine was collected through a catheter placed in the vesica. The experimental period proper was preceded by a relatively long period in which the sugar was administered in order to obtain constant concentrations of hexose and creatinine in blood and urine. Thus the significance of the renal "dead space" was minimized.

Poisoning with phlorizin was established in the large majority of the experiments by means of intravenous injection of a solution containing 5 % phlorizin and 2.5 % NaHCO<sub>3</sub>. The phlorizin was given in doses of 0.2—0.3 gr. per kg. body weight. In some other cases phlorizin poisoning was effected by daily intramuscular injections for three to five days prior to the experiment; in these cases the phlorizin was suspended in oil.

In the young healthy men experimented upon the hexoses were given in some cases orally, in others by constant venous injection during several hours. In order to secure a suitable diuresis 0.5 to 1 litre of water was given orally per hour.

The blood analyses were all performed on oxalated plasma.

#### *The glomerular filtration rate:*

Determinations in the animal experiments were made with the aid of creatinine given subcutaneously or intravenously. Creatinine in plasma and urine was determined according to REHBERG and HOLLEN

(1931). In man, inulin was used as a measure of filtration. The inulin content of plasma and urine was determined after the removal of glucose and fructose by adsorption to yeast. The inulin was hydrolyzed by acid to fructose, which was determined according to ROE (1934).

As the creatinine clearance was used as a measure of glomerular filtration in the cat, it was necessary to demonstrate the identity of inulin clearance to creatinine clearance in this animal. The ratio between inulin clearance and creatinine clearance in three cats is shown in fig. 1. The experimental periods included creatinine-plasma concentrations from 3.7 mgm% to 51 mgm%, and plasma-inulin concentrations from 4.6 mgm% to 96 mgm%. The diuresis varied from 0.02 c. c. per minute to 0.77 c. c. per minute.

*Glucose:* In both plasma and urine the HAGEDORN, NORMAN JENSEN method was used. After dilution of the urine with equal parts of distilled water, alcohol 10 % and acetic acid 1 %, the non-carbohydrate reducing substances were removed by adsorption to purified carbon, as modified after ROE (1934) and KRARUP (1935).

*Fructose* was determined according to ROE (1934) on Somogyi-filtrates in plasma and on urine after adsorption with purified carbon. The intensity of the colour developed by fructose and the resorcin reagent was determined by means of a Dubosq' colorimeter. The disturbing colour caused by glucose or galactose in the samples was corrected for by using fructose solutions with contents of glucose or galactose equal to those of the plasma and urine samples. By this procedure the fructose content was determined with sufficient accuracy (the error was found not to exceed  $\pm 2\%$ ).

*Galactose:* The determinations were carried out by means of the HAGEDORN, NORMAN JENSEN method after glucose and fructose had been removed from the plasma filtrates by adsorption to living yeast according to LARSEN and SVEINSSON (1940).

In determinations of the galactose content in urine the non fermentable reducing substances (including creatinine) except galactose were removed by adsorption to purified carbon. Then the fermentable sugars, glucose and fructose, were adsorbed to living yeast and the galactose concentration determined by HAGEDORN, NORMAN JENSEN.

In plasma samples containing fructose, galactose, creatinine and glucose the three former substances were determined separately; the

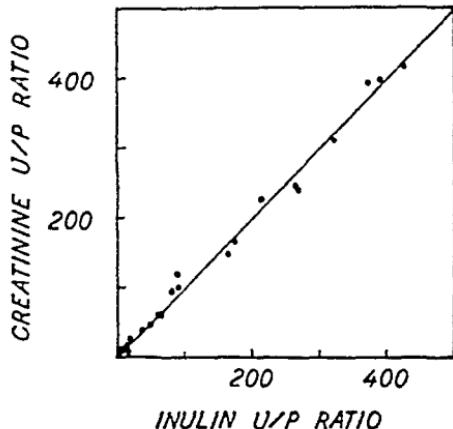


Fig. 1. Relation between Inulin and Creatinine U/P ratio; from experiments on four cats.

glucose content was calculated as the difference between total reduction and the reducing power of the three former substances.

The determination of galactose in plasma and urine is more inaccurate than that of fructose or glucose; the results obtained from determinations on solutions with known amounts of galactose varied  $\pm 5\%$  from the real values.

In the experiments on man samples of venous blood were used. As fructose is assimilated by the tissues it was necessary to ascertain that the arterio-venous difference of this monosaccharide is of no significance in these experiments.

The figures from experiments on 10 persons are given below in Table I. Samples of arterial blood were obtained by puncture of the brachial artery.

Table I.

*Simultaneous Fructose Concentrations in Arterial and Venous Plasma Samples.*

Artery mgm. per 100 c. c.	Vein mgm. per 100 c. c.	Difference A—V
2.5	2.7	— 0.2
3.4	3.2	+ 0.2
5.7	4.7	+ 1.0
8.2	7.9	+ 0.3
8.3	7.1	+ 1.2
10.8	10.0	+ 0.8
13.4	12.5	+ 0.9
14.1	13.6	+ 0.5
21.7	20.1	+ 1.6
34.3	32.2	+ 2.1

### 1. Fructose and Galactose as Threshold Substances.

The classical threshold substance is glucose. Glucose may be excreted with an U/P ratio below 1 and therefore must be absorbed by the tubular cells by an active mechanism. In glucose the reabsorptive mechanism is very efficient, and relatively high plasma concentrations of glucose may be maintained without any appreciable elimination in the urine. The demonstration of an U/P ratio of fructose and galactose below unity is taken as a sufficient criterion of their character as threshold substances.

Former experiments on the renal elimination of fructose and galactose have not brought out any evidence for these monosaccharides as threshold substances. From various experiments on fructose elimination by DE LUCIA and CLAAR (1934), STERKIN and WENGEROWA (1934), and FISZEL and WIERZUCHOWSKI

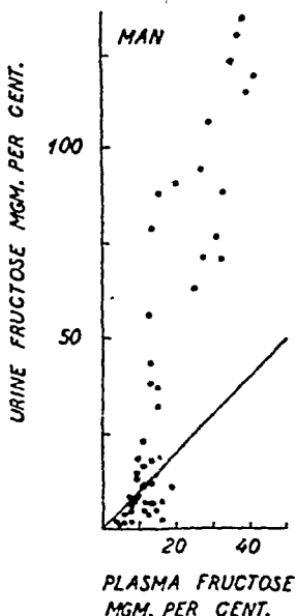


Fig. 2. Relation between urine and plasma concentrations of fructose from experiments on 18 persons. The oblique line indicates an U/P ratio equal to 1.

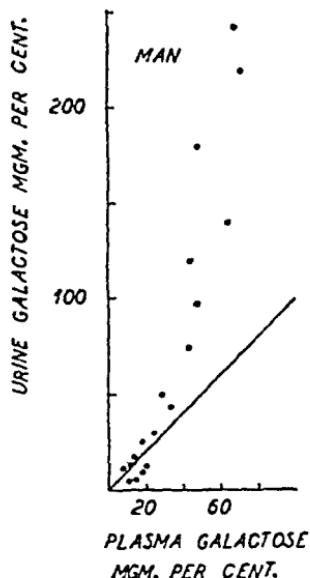


Fig. 3. Relation between urine and plasma concentrations of galactose from experiments on 2 persons. The oblique line indicates an U/P ratio equal to 1.

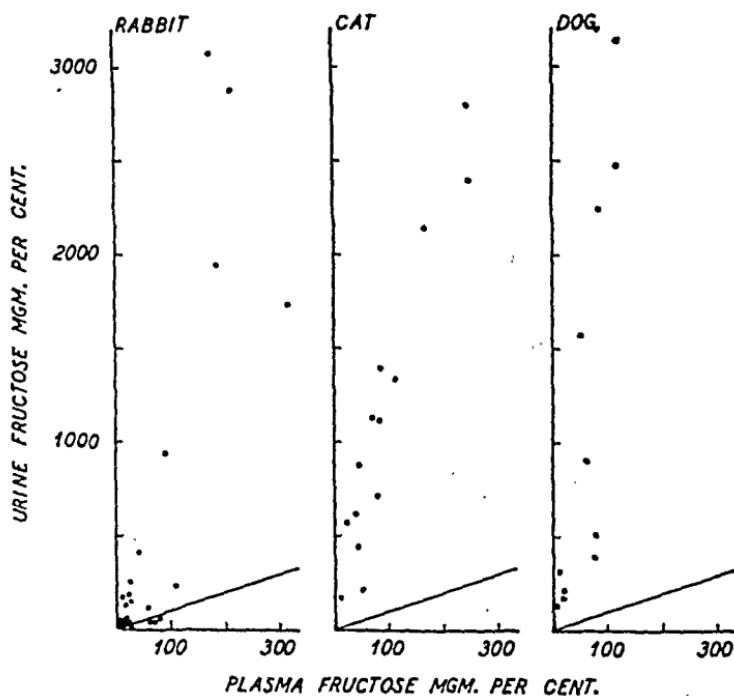


Fig. 4. Relation between urine and plasma concentrations of fructose in rabbit, cat and dog. The oblique line indicates an U/P ratio equal to 1.

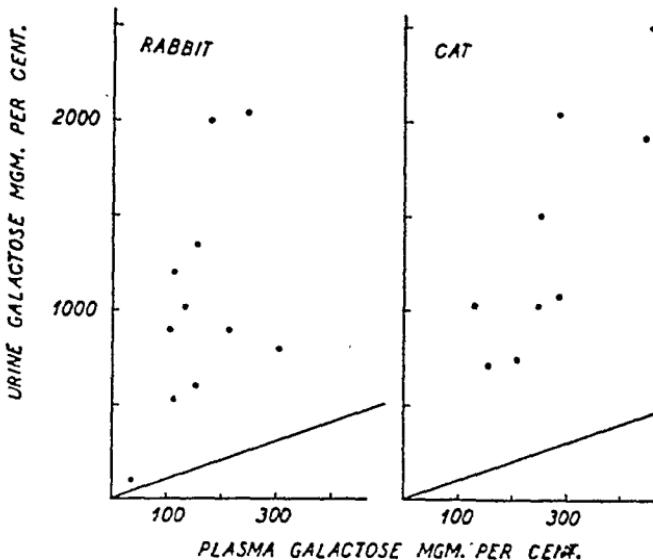


Fig. 5. Relation between urine and plasma concentrations of galactose in rabbit and cat. The oblique line indicates an U/P ratio equal to 1.

(1935) and on galactose elimination by HARDING and GRANT (1933), CHANDLER and ROWE (1924), SEKURACKI and WIERZUCHOWSKI (1935) and NISSEN (1937) it may be concluded that the threshold, if it exists at all, must be very low.

In our experiments it is demonstrated that in the human kidney fructose and galactose are eliminated with U/P ratios below 1 at plasma concentrations below 15 mg% (figs. 2 and 3). In rabbits, cats and dogs we were unable to demonstrate an U/P ratio definitely below 1 (figs. 4 and 5). From the following experiments, however, it is justifiable to assume that the renal reabsorption of fructose and galactose in these animals depends nevertheless on an active mechanism.

## 2. The Relation of Fructose and Galactose Reabsorption to the Amounts Filtered.

In order to find the ratios of the amounts of fructose and galactose reabsorbed to those filtered we determined the renal excretion at various plasma concentrations and at glomerular filtration rates as constant as possible. The results are given in figs. 6, 7 and 8, in which the amounts filtered and reabsorbed per minute are plotted out in a coordinate system. In all the animals and persons experimented upon the amounts of reab-

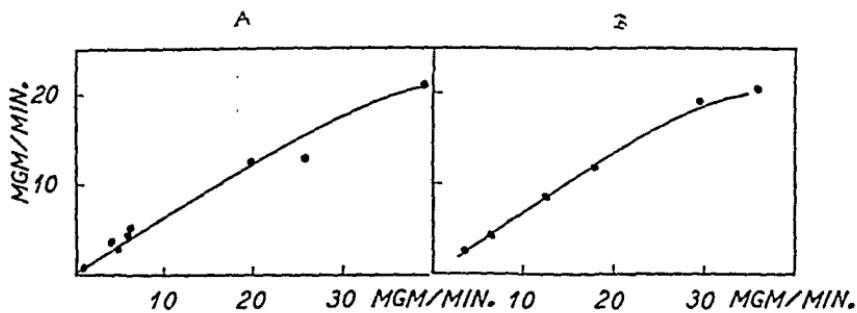


Fig. 6. Relation between the amounts of fructose filtered (abscissa) and the amounts reabsorbed (ordinate). A. in the rabbit  
B. in the cat.

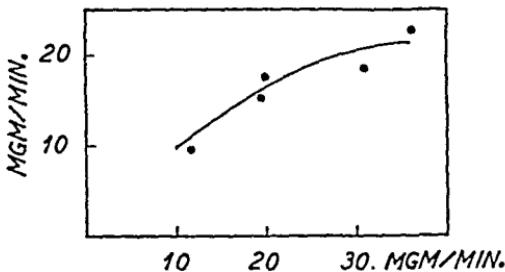


Fig. 7. Cat. Relation between the amounts of galactose filtered (abscissa) and the amounts reabsorbed (ordinate).

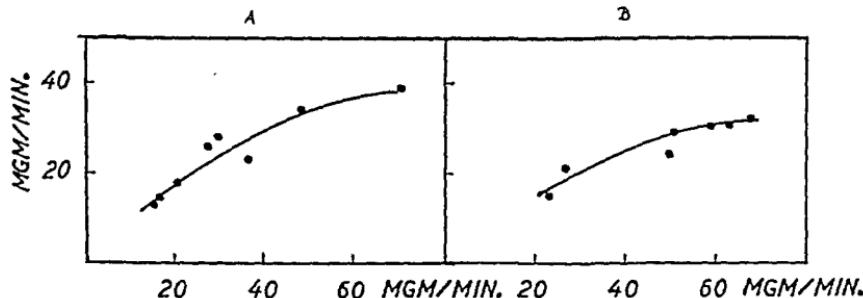


Fig. 8. Man. A. Relation between the amounts of fructose filtered (abscissa) and the amounts reabsorbed (ordinate).  
B. Relation between the amounts of galactose filtered (abscissa) and the amounts reabsorbed (ordinate).

sorbed monosaccharide were found to increase in proportion to the amounts filtered. At excessively high filtration rates, however, the rate of reabsorption of all the monosaccharides mentioned while still increasing did so at a reduced rate. When in experiments on two rabbits the plasma concentrations of fructose were increased to 700, 1,000 and 1,500 mgm% the reabsorption of fructose was not increased beyond the values obtained at about 700 mgm%.

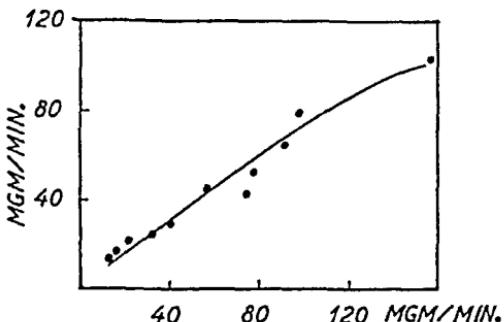


Fig. 9. Cat. Relation between the amounts of glucose filtered (abscissa) and the amounts reabsorbed (ordinate).

In their interesting experiments on dogs SHANNON and FISHER (1938) found an unchanged reabsorption when the glucose concentration in the filtrate was altered; the maximal amount of glucose reabsorbed was constant at various plasma concentrations high enough to yield a frank glucosuria. NI and REHBERG (1930), on the other hand, hold the view that reabsorption is increased with increasing plasma concentration, the reabsorbed quantity being dependent on the difference in concentration between plasma and tubular fluid.

### 3. The Effect of Increased Diuresis, Phlorizin Poisoning and Saturation of the Glucose-Absorptive Mechanism on the Reabsorption of Fructose and Galactose.

In order to find out to what extent the reabsorption is due to an active process the following investigations were made:

#### a) Reabsorption under Variations in Diuresis.

Fructose and galactose being hexoses, it is assumable that, as glucose is absorbed actively, diffusion through the tubular cells is doubtful. As in the case of the typical threshold substance glucose, it was possible to demonstrate that fructose reabsorption is fairly independent of variations in the water excretion. This appears from experiments on a cat, a rabbit and a dog, in which fructose was administered at the same time as the diuresis was increased by intravenous injection of sodium sulphate. Only when the diuresis is increased so much that the U/P ratio for creatinine approaches 1 is there a reduction of the fructose

reabsorption whereby the fructose/creatinine ratio approaches the value of 1 (fig. 10). Under these conditions, however, there is also a marked decrease of the glucose reabsorption. Urea, which is regarded as a typical non-threshold substance, will on the other hand, with a falling creatinine U/P ratio show an increasing urea/creatinine clearance ratio already at much higher U/P creatinine ratios.

Again under these conditions the three hexoses reveal no great differences in their individual reabsorption.

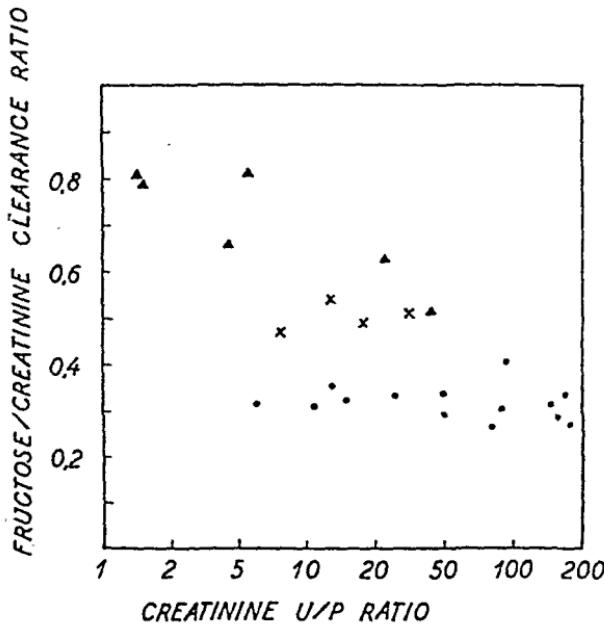


Fig. 10. The dependence of fructose reabsorption of variations in the water output. Experiments on dog (x), cat (●) and rabbit (▲) under sulphate diuresis

#### b) Reabsorption under Phlorizin Poisoning.

The demonstration that the reabsorption of fructose and galactose is sensitive to poisoning of the tubular cells by phlorizin would indicate that the reabsorption of these substances is brought about by chemical processes similar to those governing the active reabsorption of glucose.

Inhibition of fructose reabsorption by phlorizin was clearly demonstrable in rabbits and dogs in both acute and chronic poisoning (Table II and Fig. 11). The inhibition of the fructose reabsorption is reflected in the increase of the fructose/creatinine clearance ratio. On the other hand, this was not definitely de-

monstrated in the cat. Possibly this may be due to the fact that the plasma concentration of fructose increases distinctly after the application of acute phlorizin poisoning, and this makes the comparison between the periods prior to and after the poisoning difficult.

Table II.

*Influence of Phlorizin upon Renal Excretion of Fructose  
in the Rabbit, Cat and Dog.*

	Fluid		Fructose			Glucose		
	urine cc/min.	glomerular filtrate cc/min.	plasma mgm%	urine mgm%	fructose/ creatinine clearance ratio	plasma mgm%	urine mgm%	glucose/ creatinine clearance ratio
Dog No. 1. before phlor:	1.16	153	78	2,220	0.21	172	200	0.01
	1.70	198	83	3,150	0.32	0	155	0
	4.2	199	66	2,160	0.46	128	2,740	0.46
after phlor:	3.1	184	74	2,250	0.52	116	3,800	0.55
	3.7	188	100	2,700	0.81	155	5,300	0.91
Dog No. 2. before phlor:	2.4	57	109	1,351	0.52	199	0	0
	2.0	49	106	1,485	0.57	194	0	0
	4.3	53	127	1,422	0.90	227	2,168	0.77
after phlor:	3.7	46	145	1,363	0.76	201	2,137	0.85
Rabbit: before phlor:	0.07	5.0	20	224	0.15	167	0	0
	0.10	6.0	23	196	0.14	177	0	0
	0.70	10.0	23	217	0.66	207	1,470	0.50
after phlor:	0.23	4.7	22	274	0.63	193	2,860	0.74
	0.18	4.7	22	350	0.63	189	4,350	0.00
	0.13	13.9	33	2,741	0.79	388	0	0
Cat No. 1. before phlor:	0.72	8.0	74	670	0.81	377	3,640	0.87
Cat No. 2. before phlor:	0.04	8.3	35	2,961	0.44	180	0	0
	0.26	3.9	113	1,064	0.73	288	2,380	0.55
Cat No. 3. before phlor:	0.06	14.2	15	2,080	0.64	204	0	0
	0.45	8.1	27	323	0.64	263	3,577	0.75

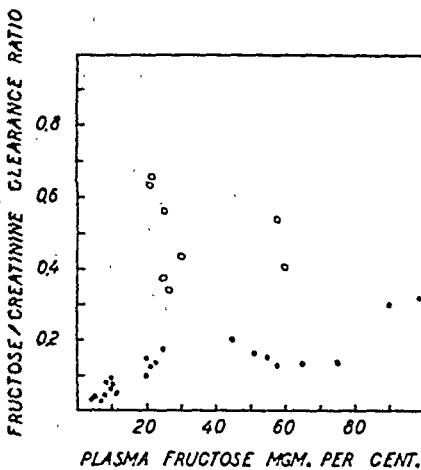


Fig. 11. Fructose reabsorption before (●) and after (○) poisoning with phlorizin. Experiments on 8 rabbits.

Table III.

*Influence of Phlorizin upon Renal Excretion of Galactose in the Rabbit, Cat and Dog.*

	Fluid		Galactose			Glucose		
	urine cc/min.	glomerular filtrate cc/min.	plasma mgm.%	urine mgm.%	galactose/ creatinine clearance ratio	plasma mgm.%	urine mgm.%	glucose/ creatinine clearance ratio
Dog:								
before phlor:	3.2	190	162	2,100	0.42	193	0	0
	3.5	193	171	2,000	0.40	176	0	0
after phlor:	5.8	220	266	3,160	0.69	114	1,900	0.34
	5.4	193	201	3,100	0.84	123	2,100	0.53
chronic pois. with phlor:	5.9	145	110	2,580	0.92	125	2,400	0.80
Rabbit:								
before phlor:	0.12	6.9	86	1,200	0.24	88	0	0
after phlor:	0.60	6.1	112	645	0.57	122	930	0.59
Cat No. 1:								
before phlor:	0.26	4.2	141	650	0.28	202	0	0
after phlor:	0.58	3.1	217	600	0.52	165	720	0.81
Cat No. 2:								
before phlor:	0.07	5.8	202	3,480	0.22	200	0	0
after phlor:	0.36	4.5	253	1,160	0.37	268	2,920	0.38

The reabsorption of galactose was inhibited by both acute and chronic poisoning of the tubular cells of rabbits, cats and dogs. (Tab. III). It appears from all the experiments that total inhibition of glucose reabsorption was not induced, even in the case of chronic poisoning with phlorizin by daily injections over a period of six days. This may perhaps also explain the uncertain result obtained in the experiments with cats (Table II).

As regards the three hexoses the mechanism of reabsorption is sensitive to phlorizin poisoning. The degree of sensitivity differs for each of the three hexoses.

c) **Reabsorption during Saturation of the Hexose-Reabsorptive Mechanism with Glucose.**

Active absorption must be assumed to have a certain limited capacity, wherefore it ought to be possible to test the specificity of the reabsorption mechanism to the various monosaccharides by determining the reabsorption of one monosaccharide before and after saturation of the absorption mechanism with another.

Results from an experiment of this kind are given in Table IV. Attempts to saturate the absorption mechanism with glucose (by increasing the plasma-glucose concentration) led to a distinct reduction of the reabsorption of both fructose and galactose in cats. In man, too, there was a reduction of the reabsorption of fructose after an increase of the plasma-glucose concentration. On the other hand, no such demonstration was possible with the rabbit.

Total saturation of the reabsorption mechanism was not achieved. As is shown in Fig. 9 the reabsorption of glucose increased steadily with augmenting quantities of glucose filtered. This may probably explain the negative result with the rabbit.

These results show that the mechanism of reabsorption for the three hexoses, glucose, fructose and galactose, has certain factors in common, whereby the reabsorption of one hexose may under certain circumstances be affected by a simultaneous reabsorption of another.

Table IV.

*Influence of Increased Glucose Reabsorption upon Tubular Reabsorption of Fructose and Galactose in the Cat and upon Reabsorption of Fructose in Man.*

	Fluid		Fructose			Glucose		
	urine cc/min.	glomerular filtrate cc/min.	plasma mgm%	urine mgm%	fructose/ creatinine clearance ratio	plasma mgm%	urine mgm%	glucose/ creatinine clearance ratio
Cat	1 0.13 0.45	7.3 7.0	47 47	1,330 555	0.50 0.78	237 475	0 2,625	0 0.25
	2 0.09 0.46	8.0 7.9	42 45	1,020 329	0.28 0.42	175 512	0 2,355	0 0.26
	3 0.25 0.68	17.6 11.1	91 77	1,404 456	0.22 0.36	256 774	0 2,080	0 0.17
	4 0.10 1.06	16.4 14.2	14 16	1,302 171	0.55 0.79	176 549	0 2,470	0 0.33
Man	1 19.0 20.1	126 143	23 20	7 16	0.05 0.14	93 272	0 198	0 0.10
	2 22.5 22.9	141 157	12 12	6 14	0.09 0.17	75 193	0 61	0 0.05
	3 11.7 27.8	146 137	33 36	122 130	0.29 0.74	82 271	0 514	0 0.28
	Fluid		Galactose			Glucose		
Cat	1 0.07 0.40	5.7 4.8	202 250	3,480 1,940	0.22 0.77	200 442	0 2,180	0 0.41
	2 0.07 0.39	21.0 18.7	288 316	22,700 7,420	0.23 0.49	190 623	0 7,330	0 0.25

### Discussion.

It has been demonstrated beyond doubt that the hexoses, fructose and galactose, are like glucose reabsorbed actively in the kidneys. On the other hand it has been more difficult to decide whether there is a common mechanism of reabsorption for the three hexoses. The differences in the threshold values of the three hexoses suggests very definitely that the reabsorbing cells in the tubules are capable of distinguishing between these monosaccharides. If we assume that an intermediate phosphoryla-

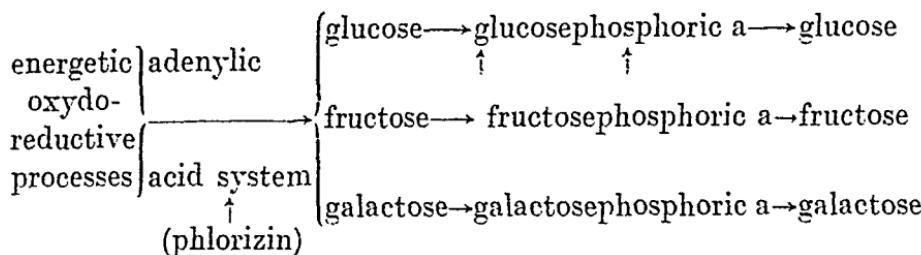
tion and dephosphorylation of the hexose molecule itself is necessary to reabsorption the explanation of this power of the tubulus cells may be that there is a specific phosphorylating and dephosphorylating enzymic system for each single monosaccharide. In this manner the various threshold values in the kidneys for the three hexoses may well be ascribed to different activities (concentrations) of the specific enzymes.

However, the results given in the present paper indicate at the same time a certain inter-relation between the reabsorption of the various hexoses. This mutual dependence might be explained by its being the same energy process on which the phosphorylation of all three hexoses depends.

The difference in the reabsorption of the three hexoses in the tubules, which as already stated is presumably associated with the specific phosphorylating and dephosphorylating enzymic systems, may lie either in the initial phosphorylation or in the concluding dephosphorylation. Whereas the phosphorylation of the three hexoses consists of a conversion of each hexose into the corresponding phosphoric acid ester, dephosphorylation may proceed partly as a direct breaking down of the hexose phosphoric acid to the corresponding hexose, and partly as conversion of the phosphate esters of fructose and galactose to glucose-phosphoric acid, which is thereafter dephosphorylated to glucose. It is possible that such a conversion of fructose and galactose governs the difference between the reabsorption of these hexoses and glucose. A conversion of this kind has been demonstrated for fructose. However, it would seem unreasonable to assume that all the reabsorbed fructose and galactose should be converted into glucose by the passage through the tubules. Fructose can be phosphorylated both in carbon-atom No. 6 as in carbon-atom No. 1. The 6-ester may be converted into glucose-6-phosphoric acid and dephosphorylated to glucose, whereas the 1-ester is dephosphorylated direct to fructose. The fact is that fructose-1-phosphoric acid is not affected by the phosphohexomutase demonstrated by LOHMANN, which governs the conversion of fructose-6-phosphoric acid into glucose-6-phosphoric acid. There may be a similar two-way dephosphorylation of galactose.

Poisoning with phlorizin has proved to attack the reabsorption of all three hexoses. This phlorizin inhibition must therefore interfere with a common link of the reabsorption mechanism of the hexoses. The common link — on the assumption that the

phosphorylation theory is correct — must be either the energy processes that actuate the phosphorylation, or the transfer of phosphate that takes place by means of the common phosphate donator (see schema), the adenylic acid system.



However, phlorizin does not primarily affect the oxydative processes in the organism. The sensitivity of the reabsorption of the three hexoses to phlorizin may depend on an inhibition of the phosphate transfer through the adenylic acid system.

In experiments to saturate the reabsorption mechanism with glucose it was demonstrable with some measure of certainty that when several hexoses are reabsorbed simultaneously there is a mutual competition between them. After the above schema this competition may be regarded as a rivalry to act as phosphate acceptor to the common phosphate donator, the adenosinetriphosphoric acid. It would seem rather improbable that the reabsorption of one hexose should be completely blocked by the full saturation of the reabsorption mechanism with another. SHANNON (1938) claimed to have demonstrated this for xylose, the reabsorption of which was completely inhibited by increasing the filtered amount of glucose to glucosuria. This finding is difficult to reconcile with the hypothesis outlined above.

### Summary.

Fructose and galactose were demonstrated to be threshold substances, by which is meant that they are both reabsorbed actively by the renal tubular cells.

A kidney threshold for fructose and galactose was demonstrated for man only (U/P ratio below 1).

The U/P ratio for fructose and galactose, which at plasma concentrations between 15 and 20 mgm. per cent. did not exceed 1, rose very steeply at plasma concentrations above these values.

In rabbits, cats and dogs the active reabsorption of fructose and galactose was demonstrated by:

1. the indepence of fructose reabsorption of variations in the excretion of water.
2. the sensitivity of the mechanism of reabsorption to poisoning of the tubular cells with phlorizin.
3. experiments in saturating the mechanism of reabsorption for glucose (hyperglycaemia), which reduced the reabsorption of simultaneously filtered fructose and galactose (cat, man).

The reabsorption of fructose and galactose was found to increase in proportion to increasing amounts filtered. In the experiments on the cat creatinine was employed for filtration determinations after the identity between creatinine and inulin clearances in this animal had been established.

The results obtained appear to agree with the theory of an intermediate phosphorylation and dephosphorylation during the reabsorption of glucose, fructose and galactose in the kidneys. In regarding the adenylic acid system as a common phosphate donator to these three hexoses, the specific kidney threshold for each of the three hexoses is explained by the assumption of specific phosphorylating and dephosphorylating enzymic systems for each hexose in the cells of the tubules.

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ACTA PHYSIOLOGICA SCANDINAVICA  
VOL 6, SUPPLEMENTUM XVII

*From the Chemical and Histological Departments,  
Karolinska Institutet, Stockholm*

PROTEIN METABOLISM  
IN THE NERVE CELL DURING  
GROWTH AND FUNCTION

BY

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STOCKHOLM 1943



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## Introduction.

The tissue of the central nervous system is composed, broadly speaking, of nerve cells, nerve fibres and special connective tissue. There is no possibility of reliably preparing these various components separately from one another, for the purpose of macrochemical analysis. An analysis of the actual cell, in which the primary processes of growth and function are carried on, has, therefore, not been found practicable with the usual chemical methods, except on an extremely limited scale.

In view of these circumstances, the author has made it his aim to study the nucleotide and protein metabolism in individual nerve cells with the aid of special cytochemical methods. The task to which he thus addressed himself was to examine details of about  $1 \mu$  in magnitude in a detached cell, to determine its chemical composition and to compute its content of substance. The amounts of substance with which we are here concerned lie within the order of magnitude of  $10^{-9}$  mg, whilst the lower limit for ordinary micro-chemical methods for biological purposes, in the most favourable cases, scarcely exceeds  $10^{-3}$  mg. Methods which admit of the determination of nucleotides and protein substance in cell parts of that magnitude, and which are based on the specific absorption of those substances in ultraviolet, have, however, been elaborated by the Swedish investigator, CASPERSSON.

As an important detail in these studies, special attention has been devoted to the distribution in the nerve cells of proteins rich in hexone bases. That such proteins appear in considerable amounts during breaking-down and rebuilding processes in tissues had already been made clear by the macro-chemical investigations of MIESCHER, KOSSEL and SCHENK. Studies by CASPERSSON show their participation in the formation of proteins in the individual

cell. With the absorption-measurement methods just referred to, however, their distribution in the cell can be estimated only approximately. With a view to greater precision, the author of this paper has devised a method for determining the content of free amino-groups in individual cell parts by measuring the capacity of those groups for binding dyes. The preliminary results of such tests will be reported in the following pages as an item in this investigation.

The first part of this paper deals with the nucleotide and protein metabolism during the development of the nerve cell. The second is concerned with investigations of adult nerve cells as a background for the following studies of metabolism processes during different functional conditions. The third treats of the nucleotide and protein metabolism in motor and sensory nerve cells during states of irritation. The fourth reports certain studies of changes in nerve cells after nerve excision and the following regeneration. The concluding part contains an account of attempts made, on the basis of Purkinje cells, to apply the results of the above specified studies to normal conditions.

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## P A R T I.

### CHAPTER I.

#### The Protein-forming System in the Cell.

Possibilities for studying the principal structure-forming substance in a cell, namely the nucleic acids and proteins, and for obtaining some insight into their metabolism in the individual cell have been afforded by the method elaborated by CASPERSSON. (See Chapter II, Part I. For particulars regarding the methods employed and for the literature of earlier macro- and micro-chemical investigations, the reader is referred to the studies of CASPERSSON 1936—1940). Investigations with these methods on different material have shown that *the nucleic acids are essential factors in the reproduction of living material, in the growth of the cell and in the formation of cellular protein*.

In cytochemical studies, CASPERSSON and collaborators (1936—1942) show that *the nucleus must be regarded as the principal centre for the protein-formation in the cell*. In connection with the mitotic cell-division, the reproduction of the genes *in the nucleus* is carried on with the aid of *ribodesose nucleic acids*. When the interphase nucleus is developed, protein substances are formed from the gene-carrying elements. Also the other proteins contained in the interphase nucleus are developed during the final stage (the telophase) of the cell-division process from the chromatin. In this phase a chemical distinction between the two components of the nucleus, the euchromatin and the heterochromatin, can be observed. The euchromatic gene-carrying chromosome parts in fact produce, during this stage, proteins with “the absorption character of the higher coagulable proteins” (see below), whereas the heterochromatin produces more simply-built proteins with the absorption character of the proteins rich in hexone bases. The heterochromatic chromosome parts do not show a linear structure to the

same extent as the euchromatic areas. The nucleolus, which appears during the telophase, when the protein-formation in the cell is taking place, is characterized by as large a content of proteins rich in hexone bases as the heterochromatin. It seems probable that the major part of the nucleolar material consists of proteins produced from the heterochromatin.

Nucleotides seem also to participate in the formation of protein *in the cytoplasm*, although here they are of a different character than those in the genes-carrying elements. They consist in fact of *ribose nucleotides*.

In a series of tissues from the animal and vegetable kingdom, large concentrations of ribose nucleotides could be observed in the cytoplasm of rapidly growing cells. The connection between the cytoplasmic nucleotides and protein-formation was shown with particular distinctness in various protein-producing gland cells (CASPERSSON, LANDSTRÖM-HYDÉN and AQUILONIUS 1941). Serous gland cells in general are characterized by large concentrations of ribose nucleotides in the cytoplasm. These cytoplasmic nucleotides can be shown to constitute the major part of the ergastoplasm described in the literature. In the exocrine part of the pancreas a functional connection could likewise be shown between the content of ribose nucleotides in the gland cells and the production of proteins.

B. NORBERG (1942), with a cytochemical method elaborated by him for the determination of nucleic acid phosphorus in application to cells, has, in several important respects, confirmed the view advanced by CASPERSSON and collaborators, that the production of proteins is carried on with the aid of nucleotides. NORBERG found in rapidly growing embryonic tissues and in protein-producing glands a high content of nucleic acid phosphorus, as compared with adult tissues and glands producing but little protein. These converging results are in good correspondence with those obtained with the above-mentioned methods for absorption measurement, and show that the content of nucleic acid increases in close connection with the increase of protein in the cells.

As shown by the researches above referred to, a feature common to the heterochromatin and the nucleolus is that both contain large amounts of proteins with the absorption character of proteins rich in hexone bases. The amount of heterochromatin in the chromosome set was found to affect the amount of ribose

nucleotides in the cytoplasm of the egg-cells of *Drosophila* (SCHULTZ and CASPERSSON 1938). In fact, if an extra Y chromosome, which in the fruit-fly (*Drosophila*) is heterochromatic, was introduced in the nucleus, the amount of cytoplasmic nucleotides showed an increase. These results indicate the possibility of a connection

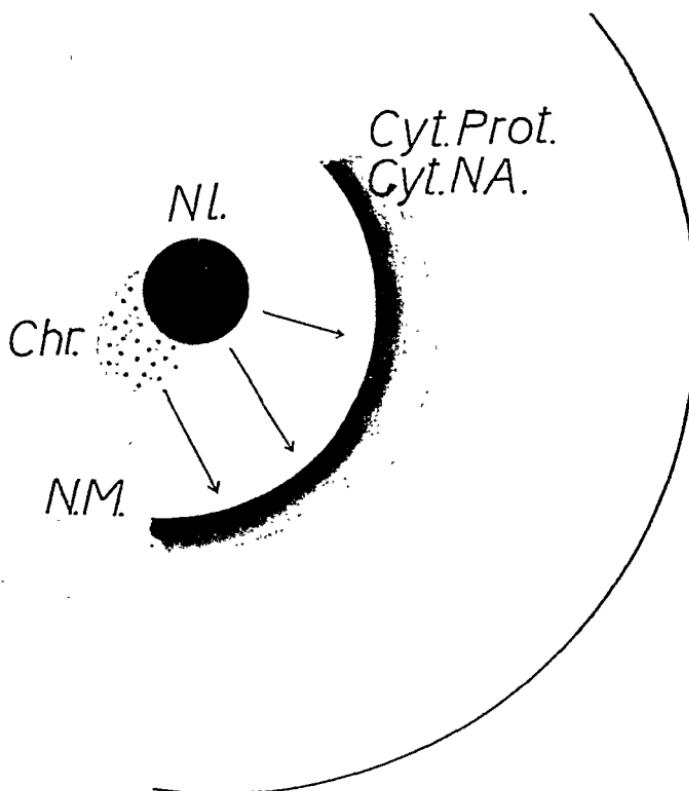


Fig. 1. Diagrammatic view of the cytoplasmic protein-forming system. *Chr* chromocenter *NL*, nucleolus. The arrows indicate the migration of proteins from the heterochromatin, direct or via the nucleolus, to the nuclear membrane, *N.M.*. In the vicinity of the latter the formation of cytoplasmic nucleic acid, *NA*, and cytoplasmic protein proceeds.

between the heterochromatic chromosome elements and the cytoplasmic nucleotides. A characteristic feature of all cells having large amounts of nucleotides in the cytoplasm, and in which a formation of proteins proceeds or has proceeded, is the existence of large, ribose-nucleotide nucleoli. This correlation between nucleoli, on the one hand, and cytoplasmic nucleotides, on the other, bears out the theory of a functional connection between the protein-forming systems of the nucleus and cytoplasm. In sea-urchin eggs the nucleotides in the cytoplasm showed the greatest accumulation round the nuclear membrane

(CASPERSSON and SCHULTZ 1941). From this fact the authors inferred that the synthesis of ribose nucleotides was carried on in the vicinity of the nuclear membrane. The same phenomenon has subsequently been observed in many types of cell. In intensely functioning gland cells, for example, large concentrations of ribose nucleotides are found close to the nuclear membrane, especially during those stages when the cell, after a copious secretion, is engaged in restoring the content of nucleotides and protein substance in the cytoplasm. These various results all point in the same direction.

In connection with a previously reported investigation of ganglion cells from certain fishes, the author studied in detail the mechanism whereby the ribose nucleotides were formed, as also the production of cytoplasmic proteins close to the nuclear membrane (HYDÉN 1943). See also Chapter I Part II.

Investigations of this nature, some of the most important of which have been briefly mentioned above, seem to have shown that *the formation of the cytoplasmic protein* is effected by the following mechanism (See the outline drawing in Fig. I). It is primarily induced by the formation of proteins rich in hexone bases, derived from the heterochromatic chromocentre area (Chr). From the nucleolus (Nl) and the chromocenter the proteins rich in hexone bases migrate towards the nuclear membrane (NM). In the vicinity of the latter a formation of ribose nucleotides and cytoplasmic proteins proceeds.

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## CHAPTER II.

### Materials and Methods.

#### Fixation and Micro-dissection.

The material consisted of both embryonic and adult material from the central and peripheral nervous system of various mammals. In the different parts of this paper the material used for each of the experiments is specified. The tissue material was fixed (1) in accordance with the freezing-drying method elaborated by GERSH (1933, 1938), and (2) in Carnoy's solution. After embedding in paraffin, the material was sliced in series in sections 5  $\mu$  thick. The sections intended for absorption measurements were treated with chloroform and absolute alcohol, after which they were transferred to a pure glycerin bath, where they were allowed to lie for 2 hours before the ultraviolet measurement. The next following section was treated in accordance with the usual histological technique, for staining with toluidine blue — erythrosin. For the impregnation of the neurofibrils, the method elaborated by BODIAN (1936), which renders it possible to impregnate sliced material, was adopted.

The sections for absorption measurements were photographed in an ultraviolet microscope according to KÖHLER at 2570 Å, and a suitable specimen for measurement was selected. The cells picked out for this purpose were those where the section showed the central part of the cell, including the bulk of the nucleolus. This was controlled on adjacent sections. Unless otherwise stated, the photographs reproduced in the following pages are pictures of unstained sections, taken with an ultraviolet microscope at the wave-length 2570 Å. Objectives: monochromates according to KÖHLER and von ROHR, corrected for that wave-length. The aperture value shows the numerical value of the objective. Illumination of the object according to KÖHLER's rules. Plates:

Agfa standard plates. Developer: Hydroquinone 5 minutes.  
Copied on Agfa Lupex Normal printing paper.

Previous to the absorption measurement the cell was freed on one side from surrounding tissue, in order to produce an absorption-free zone close to the cell during the ultraviolet measurement. For an example see Fig. 8 a and b.

The dissection was made with a Zeiss sliding microtome or by hand with glass needles.

**Absorption measurements.** The absorption measurements in ultraviolet were made with the apparatus designed by CASPERS-SON for measuring cell parts within this wave-length range. The apparatus and the chemical details are described in studies published by that author in 1941 and 1942. With these appliances, an absorption spectrum of an object down to  $1 \mu$  in magnitude can be measured in ultraviolet light. From the absorption spectrum thus obtained the content of ultraviolet absorbing substance can be computed.

Within the central ultraviolet range there are two groups of cell substances, namely the nucleic acids and the proteins, which so dominate the absorption, that, in comparison therewith, the absorption of other substances is negligible. The nucleic acids are characterized by intense absorption with a dominating band at 2600 Å, which is due to the pyrimidin ring included in the bases of the nucleotides. The proteins have an absorption band at a somewhat longer wave-length, which is mainly attributable to the component amino-acids, tyrosin and tryptophan.

The higher coagulable proteins have an absorption maximum at 2750—2800 Å. Proteins having an absorption band within that range will be grouped in the sequel under the term "proteins with the absorption type of the higher coagulable proteins".

In the case of proteins rich in hexone bases, the tyrosin absorption band, on acid reaction, is shifted somewhat towards the long-wave range: this displacement can be reliably determined by curve analysis.

In view of misleading reports in certain publications in the English language, it must be strongly emphasized that it is the *tyrosin*, and not the protein, maximum that is first shifted. As a consequence of this shifting, the protein absorption maximum is likewise shifted towards longer wave-lengths, but in a much lesser degree. The tyrosin absorption maximum is in fact shifted to wave-lengths up to somewhat above 2900 Å.

The consequent shifting of the protein maximum is quite moderate, rarely exceeding 2850 Å.

In short, such shifting of the protein absorption maximum as may thus occur is usually so insignificant that rather precise methods are required for its determination. However, with the photo-electric methods here adopted, in which the measurements are precise to within 1 per cent, the accuracy is quite sufficient. As the mechanism for the shifting of the absorption band is not known in detail, the facts which serve as a basis for viewing the shifting of the maximum as being due to a high content of hexone bases will be briefly set forth below.

1) Absorption spectra of certain cell parts, taken between 2600 and 3000 Å, in acid or neutral reaction, cannot, like those taken on alkaline reaction, be shown to be a sum of the tyrosin, tryptophan, phenyl alanin and nucleotide absorptions. On the other hand, they may be explained by the assumption that in the proteins the tyrosin maximum, on acid and neutral reaction, lies towards longer wavelengths than in the free amino-acids under similar conditions. Such a hypothesis would also serve to explain several other observations previously reported in the literature. Weighty arguments may in fact be adduced for the view that this shifting is due to changes in the dissociation conditions of the hydroxyl group, caused by adjacent basic groups (see *loc. cit.*). A far less probable explanation is that the shifting of the absorption maximum of the proteins rich in hexone bases is due to the action of the nucleic acids present. This possibility cannot forthwith be ruled out, seeing that in all the hitherto investigated cases of a shifting of the maximum, nucleic acids had been present. It should be noted, however, that the maximum of the higher proteins, which are poor in hexone bases, does not seem to be affected at all by the presence of nucleic acids.

2) This shifting of the absorption maximum is observed with particular distinctness in embryonic material at stages where earlier macrochemical analyses of amino-acids had throughout shown a particularly high content of hexone bases in the proteins (CASPERSSON and THORELL 1941).

3) The cell areas in which this shifting of the maximum is well-marked have in all cases shown a remarkably great affinity for acid dyes, which, of course, is a clear indication that these areas are rich in basic groups.

4) Sample tests with a limited number of different preparations of basic proteins (all containing low, but varying, amounts of nucleic acids) with ordinary spectrographic methods, gave results in complete conformity with cell observations.

Seeing that the detailed mechanism for the shifting of the absorption maximum has not yet been made clear, and as

its effect is rather slight, this phenomenon, in previous studies, has been made use of solely in order to ascertain whether a cell part is distinguished by particularly large amounts of hexone bases or not, and no attempts have been made to obtain quantitative data from such tests. In view of these circumstances, the author has endeavoured to devise a preliminary method for determining the amount of basic groups in the cell protein (see Chapter II). All the cell material examined in this study has been treated in accordance with this method, which is based on an analysis of the capacity for binding dyes, in highly acid reaction. In important typical cases the intensity of the dye-binding capacity has been determined with absorption measurements, but for the most part it has been considered sufficient, at any rate for the time being, to make merely a visual estimate. It should be pointed out at once that in all the material studied in accordance with this method, high contents of hexone bases have been observed when the ultraviolet spectra had indicated a distinct shifting of the tyrosin band. This conduced to bear out the view that the shifting of the absorption maximum has some causal connection with the content of hexone bases. This being so, the question regarding the physical mechanism of that shifting seems to be of quite subsidiary importance.

As the protein and nucleotide absorption between 2500 and 3000 Å completely dominates that of the other cell substances, an absorption spectrum from a cell detail within this range will be made up of the following components:

1. The nucleotide absorption band at 2600 Å, due to the pyrimidin ring, and their non-selective absorption, which increases as the wave-lengths fall.

2. Proteins which have a tryptophan band at 2750 Å and a tyrosin band at 2750—2950 Å. Above 2600 Å, other amino-acids have a faint absorption of non-selective character, which below 2500 rapidly increases.

3. A general non-selective absorption, due to the light scattering in the preparation, and which, approximately, is in inverse proportion to the fourth power of the wave-length. Its height is determined chiefly by the manner in which the preparation is fixed. Above 2600 Å this absorption as a rule is but little marked. In most of the measurements reported here, chief importance has been attached to the comparative determinations and, as the serial measurements have not been extended much beyond

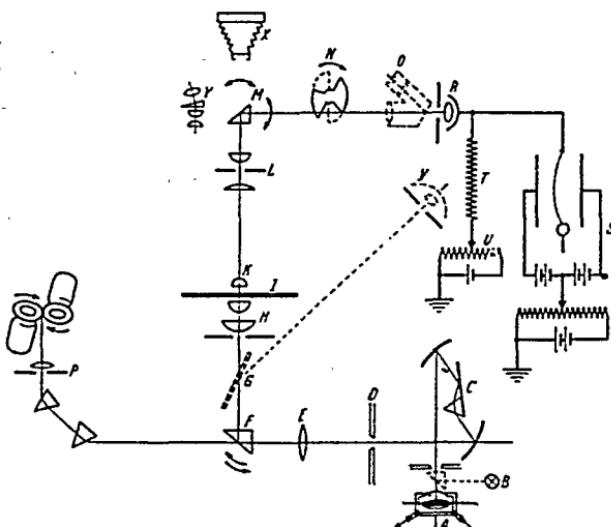


Fig. 2. Outline of construction of apparatus for taking absorption measurements in ultraviolet light of cell parts in microscopic sections. A. Water-cooled high-pressure mercury lamp, B. tungsten lamp, C. monochromator, D. exit slit, E. auxiliary lens, F. total-reflecting prism, G. semi-transparent plate, H. condenser, I. object, K. objective, L. ocular system, M. movable prism, N. rotating sector adjustable with great precision while rotating, O. centering device, R. photocell, S, T and U. main coupling for string electrometer, V. auxiliary photocell which, with the aid of G, serves to correct variations in the source of light, P. rotating spark gap.

The measurement procedure is, broadly, as follows. The object I is illuminated with monochromatic ultraviolet light from A or P. Through the quartz system K, L, M, the picture is thrown on R, whereupon the electrometer makes a deflection. The object is removed by means of a special apparatus, after which the amount of light falling on R is reduced by means of N until the electrometer gives the same reading as before. The loss of light in N is then the same as in the object.

The procedure is repeated in any desired number of wave-lengths.  
(After CASPERSSON).

3000 Å, an exact analysis has not been practicable. In such cases the assumption has been made that the loss of light at 3000 Å is due in equal measure to simple light reflection and to Rayleigh's light scattering, and the losses of light below 3000 Å have been estimated on this basis.

It is, in short, possible, from the absorption curve of a cell part, to determine the occurrence and content of nucleotides, tyrosin and tryptophan, as well as to estimate whether the tyrosin proteins, broadly speaking, are highly basic or not.

Among the other substances which contain pyrimidin groups, and which may conceivably occur in nerve cells and thus contribute to the absorption roundabout 2600 Å, vitamin B<sub>1</sub> and cocarboxylas, as well as oligonucleotides containing adenine, appear to be the most important. The preparation of the tissue sections

for ultraviolet absorption measurement is, however, of such a nature that the major part of those substances is bound to be dissolved. Moreover, the macro-chemical investigations of nerve tissue show such low contents of these substances that they could not account for more than a minor amount of the total absorption, even on the assumption of a distribution in the nerve tissue in favour of the nerve cell.

For convenience in comparing the data under discussion, the amounts of tyrosin and tryptophan obtained in the absorption measurements have in some cases been reckoned on the basis of a protein of "standard type". As the content of tyrosin and tryptophan in most of the cell proteins analyzed was found to range between 4 and 8 and 1 to 2 per cent, respectively, an average content of 5 per cent tyrosin and 1.5 tryptophan has been adopted. In the sequel, protein of such composition will be termed "standard protein".<sup>1</sup>

The construction of the apparatus and the procedure for taking an absorption spectrum of a cell detail is indicated by Fig. 2. Figs. 3 a and b show outline views of the measuring apparatus (For details see CASPERSSON 1940). The order of magnitude of the minutest object measurable in accordance with this method is about 1  $\mu$ .

### **Importance of Fixation for the Absorption Measurements.**

The selection of fixing agents was found to be of particularly great importance in the investigations in accordance with the above indicated methods of procedure. As already pointed out, material fixed in accordance with Altmann-Gersh's freezing-drying method was used for the major part of the absorption measurements in ultraviolet light. Carnoy-fixed and formalin-fixed material was also employed. As the agents thus used for the fixing of this material have such a marked tendency to precipitate protein, a very high standard must be set up for the results of the fixing, seeing that shrinkage will completely distort the determinations of the total amount of protein.

<sup>1</sup> The symbol  $\epsilon$  will be used in the sequel to denote the decadic extinction coefficient, where  $\epsilon = \log \frac{I_0}{I_1}$ ,  $I_0$  denoting the incident light and  $I_1$  the transmitted light.

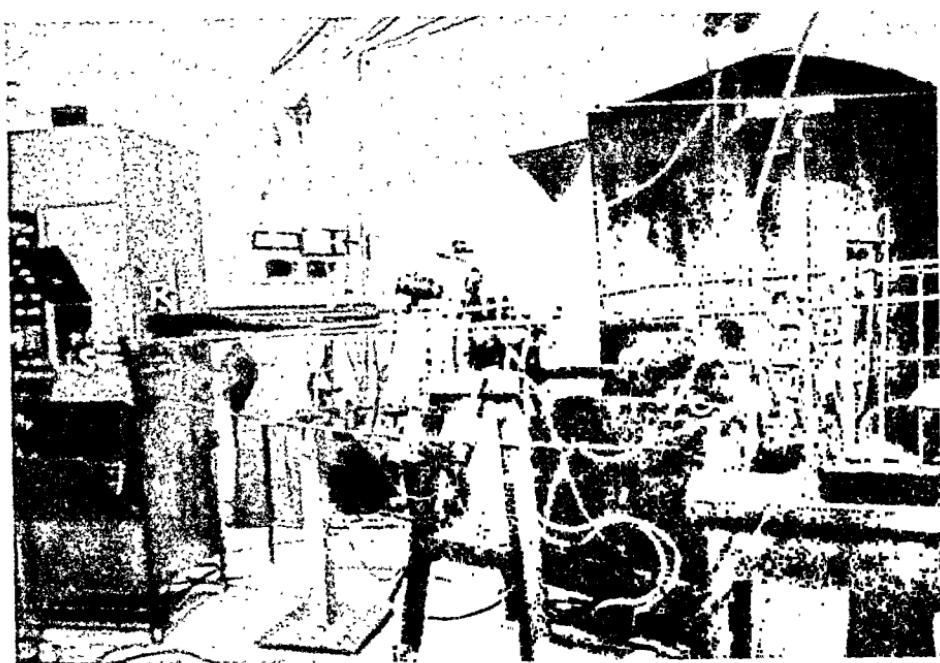


Fig. 3 a.

Fig. 3, a and b. Main set of instruments for taking absorption measurements of cell parts. From the water-cooled high-pressure mercury lamp (A), through the monochromator (C), an object placed on the stage-plate in the microscope (B) is illuminated with monochromatic ultraviolet light. To this stage is attached a shifting device (D), with the aid of which shiftings of the object, reproducible to  $0.1 \mu$ , can be effected. Through a set of microscopic lenses, made of molten quartz, a greatly magnified picture of the object, with the aid of a total-reflecting quartz prism (E), through the rotating sector (N), adjustable while in operation, is thrown on the photo-cells (R), which are enclosed in the metal box, shown to the left of the Fig. 3 b. The strength of the photo-current thereby produced is dependent on the light-absorption of the object. The current is measured with the string electrometer (S) in the metal box, and from its value one reckons the absorption.

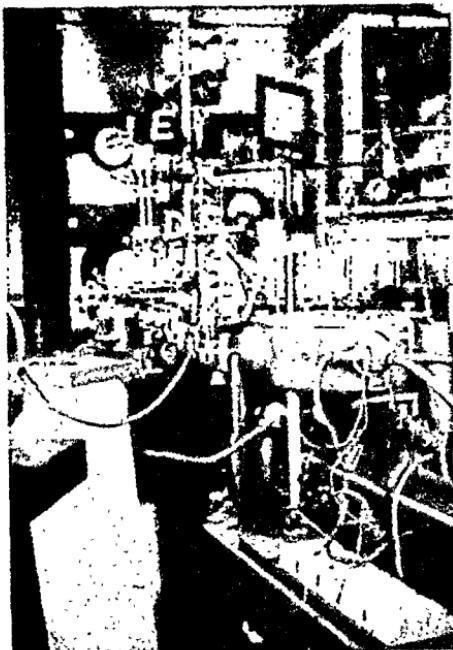


Fig. 3 b.

As above indicated, the absorption values obtained in the measurements are determined not only by the true absorption of light in the object, but also by diffuse reflection and scattering of light in its inner boundary surfaces. As regards the nerve-cell material employed, these latter factors could be eliminated by the adoption of Altmann-Gersh's freezing-drying method.<sup>1</sup> Otherwise, owing to the large content of water in the nerve tissues, this was not found possible.

The magnitude at different wave-lengths of the loss of light due to reflection and scattering can be extrapolated with the aid of absorption values between 3000 and 3500 Å. In fact, within this range, the absorption is caused, practically speaking, by those factors only. In protein objects, homogeneous in the microscope, the curve follows rather closely the fourth power of the inverted value of the wave-length, i. e. Rayleigh's formula. When the disparities *within the measurement range* are greater and begin to be noticeable in the microscope, and thus can no longer be regarded as of minor account compared with the wave-length, this statement is no longer strictly applicable. In regard to the limits see CASPERSSON (1932—33). The extreme case of infinitely large particles compared with the wave-length would give losses by reflection, which would increase but slowly as the wave-length diminishes, following the rising value of the index of refraction.

For the localization of a light-absorbing substance in a cell structure, the observation of its characteristic absorption band will suffice. Losses of light by reflection and scattering will then be of no account, seeing that the possibility of their "covering" any absorption maxima or of giving rise to "false" maxima is exceedingly small.

Ultraviolet microscopy of spinal ganglion cells cut in series and anterior horn cells shows that the aggregations of substance in the cytoplasm which absorb at 2570 Å lie uniformly scattered throughout the cell, except for the outermost zone in spinal ganglion cells. Every "measuring" ray will pass several of these accumulations of substance. The coefficient of extinction will thus be determined by the *amount* of absorbing substance which the ray passes, and will not be affected by its distribution along the path of the ray.

In the case of a very large number of the curves shown in the Figures, double measurements were taken. The divergences were

<sup>1</sup> In the sequel termed Gersh's freezing-drying method.

then found to be quite small, compared with the magnitude of the effects which were to be observed. Unless otherwise stated, the recorded absorption spectra are from material fixed in accordance with Gersh's freezing-drying method.

The factors noted above explain why the ultraviolet picture of a ganglion cell fixed in accordance with Gersh's method is entirely different from that of a cell fixed by an agent — such as Carnoy's solution — which violently precipitates protein. The cytoplasm in a ganglion cell, for example, contains larger or smaller aggregations of a substance absorbing at 2600 Å, which, when a frozen-dried cell is photographed at 2570 Å, are brought out on the plate as diffuse dark masses. (See Fig. 20.) In a Carnoy-fixed cell, on the other hand, these masses come out as more sharply defined and more deeply absorbing areas. (See Fig. 27.)

### **Localization of Ribose and Ribodesose Nucleic Acids.**

As previously indicated, with respect to the component carbohydrate, a sharp distinction has been made between ribose and ribodesose nucleic acids. In order to determine the distribution of the two types of nucleic acids in the cells, absorption spectra were first taken in ultraviolet light, whereby the distribution of nucleotides could be ascertained. Afterwards the Feulgen reaction, which shows the presence of ribodesose nucleotides, was performed on the same section. (For review of the literature on the Feulgen reaction, see MILOVIDOW 1936). In some cases adjacent sections, 5  $\mu$  thick, were used for the Feulgen reaction. As the nerve cells in question averaged 60  $\mu$  in length and 50  $\mu$  in breadth, this section contained similar structures. If the absorption measurements show large concentrations of nucleotides which do not react positively to the Feulgen test, this indicates that the nucleotides are of the ribose type.

### **Micro-incineration.**

Of the group of substances, base-pentose-phosphoric acid, which together compose a nucleotide, the two first-mentioned can be localized in cell material by the above described method. A pyrimidin or purin ring in the component bases results in an

absorption band at 2600 Å. Among the carbohydrates present, ribodesose gives a positive Feulgen reaction. The distribution of ribose nucleotides is obtained by a combined test, consisting firstly of absorption measurement in ultraviolet light and secondly of the Feulgen reaction. Possibilities for a rough estimate of the distribution in the cell also of the remaining group in the nucleic acid molecule are afforded by micro-incineration. The procedure was elaborated by POLICARD and SCOTT. In regard to the literature see A. ENGSTRÖM (1943). As the content of phosphoric acid in the nucleic acid amounts to 25 per cent, cell parts rich in nucleic acid may be expected to yield a particularly large residue of ash in micro-incineration, as compared with other parts of the cell. Micro-incineration was performed at 500° on the section which had been previously used for ultraviolet absorption measurement. In some cases, however, the adjacent section was used, instead.

## Determination of Capacity for Binding Dyes.

Many attempts have been made to draw conclusions from the results of the histological staining methods in regard to the chemical composition of, and the rebuilding processes in, cells. A copious literature on this subject shows the unreliability of those staining methods. See, for example, MÖLLENDORFF (1927), LISON (1937) and RIES (1938). MÖLLENDORFF, who has dealt very thoroughly with these problems, contends that no conclusions whatsoever in regard to the chemical composition can, in general, be drawn from the histological staining. The sole exception, he maintains, consists of a "*Niederschlagsfärbung*", occurring in certain cases, which is obtained with basic dyes and is due to the precipitation of the acid nucleic-acid colloid by the penetrating dye. MÖLLENDORFF does not go further than this concession, and declares his view that histological staining merely enlightens us regarding the density of the structure and, in certain cases, as to the way in which it is charged.

E. HAMMARSTEIN (1924) showed that the nucleoproteids had the character of a salt between nucleic acids and protein. Proceeding from this observation, E. and G. HAMMARSTEN and T. TEORELL (1928) studied the reaction between dyes and known nucleic-acid-protein compounds under uniform and reproducible conditions. The results of this investigation

showed, *inter alia*, that very minute amounts of protein completely "masked" the nucleic acid in the staining tests, whence the salts were precipitated as protein substance. These tests further indicate the general lack of specificity in the staining.

In special cases, however, the conditions appear to be more favourable. We can find in the literature a series of chemical researches which seem to show that the reaction between certain dyes and proteins in particular circumstances proceeds under stoichiometric conditions. CHAPMAN, GREENBERG and SCHMIDT (1927) determined the capacity of different protein solutions for binding acid dyes, by titration in intensely acid reaction. They found that, irrespective of the kind of dye employed, the amount of bound dye, within the error limits of the method, was equivalent to the content of free basic groups, which for the proteins, with but a slight error, may be considered to be tantamount to the sum of the free basic groups in arginin, histidin and lysin. Studies by HEWITT (1927), RAKUSIN (1928) and STEARN (1931) have yielded broadly corresponding results.

These researches show, in short, that in sample tests the binding of acid dyes by proteins, in certain circumstances, is chemical and takes place under stoichiometric conditions. As this seemed to afford a possibility for estimating the content of hexone bases in proteins even in sections, the author considered it desirable to make such dye-binding tests under uniform conditions.

### **1. Preliminary Tests for Determining the Capacity for Binding Acid Dye Groups in Cell Details.**

Sections of nerve-cell material, 5  $\mu$  thick, were stained with a dye which was soluble at pH 1 and which, on the precipitation of protein, could be bound without decolorization. Two of such dyes are ponceau RR and cyananthrol RBX. (IGEFA.) The last-mentioned dye was used for absorption measurements, whilst ponceau was employed only for visual estimates. See below.

Some of the dye is always bound in the cell structures by adsorption, thus increasing the amount of stain taken on. This adsorption of the dye increases with the duration of the staining. In order to eliminate this factor, tests were made with surface-tension reducing agents in different concentrations, with a view to determining the point at which the staining had reached a constant level. For this purpose duponol (*Dupont*) in n/10

HCl was employed. In order to determine this concentration after the elimination of stain bound by adsorption, a material in which it was possible to compute the amount of free amino-groups was selected. The material adopted to this end consisted of erythrocytes, for the reason that they are found in every tissue section, that their cross section is easily computed, and that their composition is well-known and adapted for such tests. Globin is in fact a histon-like protein (SCHULTZ 1898, O. HAMMARSTEN 1922), with a high content of hexone bases, and constitutes over 95 per cent of the total protein in the erythrocytes.

All the dye-binding tests reported in this study were made on rabbits. The content of basic amino-acids in the rabbit hemoglobin was determined in accordance with the micro-method elaborated by H. THEORELL and Å. ÅKESSON (1941). Reckoned for globin, the following results were obtained. (For purpose of comparison, the results of analyses of dog and horse hemoglobin, reckoned as globin is appended. After KEILIN and HARTREE 1937).

	In globin from rabbit hemoglobin	In globin from horse and dog hemoglobin acc. to KEILIN
Percentage arginin . . . . .	3.33 %	3.71 %
histidin . . . . .	8.87 %	8.45 %
lysin . . . . .	—	8.64 %

The direct determination of lysin is not possible with the micro-method employed; but, as other values, within the error limits of the method, correspond well with those obtained on horses and dogs, a percentage of 8.64 has been adopted also for rabbits.

Reckoned according to the free amino-groups in the basic amino-acids in globin, each individual erythrocyte in a rabbit with an average of 5,350,000 erythrocytes per mm<sup>3</sup> and 12 g Hb per 100 cc of blood (average values for rabbits according to KLIENEBERGER 1927) would bind 13.7. 10<sup>-12</sup> g of *cyananthrol*.

Staining series were arranged with different concentrations of the surface-tension reducing agent and cyananthrol, both of which were dissolved in n/10 HCl. The erythrocytes were measured photo-electrically and the amount of dye bound was computed. It was found that the most suitable concentration of the surface-tension reducing agents was about 0.1 per cent, that of the cyananthrol about 0.1 per cent. The approximately suitable staining

was obtained by this procedure after 6 hours and showed no change on prolongation. For the absorption measurements the duration of the staining was fixed at 12 hours.

The absorption measurements were made with the apparatus previously described. As a source of light a high-pressure mercury lamp was employed. For the measurements potassium-caesium photocells were used. The measurements for the sections stained with cyananthrol were taken at 5750 Å. Optics: Zeiss apochromatic lens 90, immersion, numerical aperture 1.30, ocular 10 ×, magnification in the plane of the photo-cell 5,000 ×.

As a measure of the dye-binding capacity, in comparisons between different cells and cell parts, the extinction coefficients obtained are given. In most cases it is merely necessary to compare different parts of the same section, whence a visual estimate of the intensity of the stain is sufficient. In typically important cases absorption measurements were made at the absorption maximum of the dye used. In some cases it was found necessary to compare sections from different animals. In order to obtain an additional guarantee of uniform staining conditions, the erythrocytes were computed and the values were compared.

## 2. Capacity for Binding Basic Dyes.

KELLEY (1938) studied the capacity of the cell nuclei in different tissues for taking on basic dyes, made a comparison with the content of nucleoproteids in the macerated tissue, and considered that he had found a correlation between those factors.

In order to facilitate a comparison between the distribution of the nucleotides in different cell parts, which had been determined by the above described absorption measurement procedure, and the affinity of the cell parts for basic dyes, staining was in many cases made with dyes of that type. The 5  $\mu$  thick sections were stained with a 0.1 per cent aqueous solution of toluidine blue in the presence of 0.1 per cent of duponol. The absorption measurements were carried out in accordance with the above described procedure, but at 6400 Å.

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## CHAPTER III.

# Nerve Cell Development.

### A. Literature.

Most investigations of the development of the nerve cell have been concerned in particular with the neurofibrils and the origin of neurites and dendrites. According to studies of HIS, HELD, CAJAL and others, the early forms of the development of the nerve cell are as follows:

After the germinal cell, the apolar cell, with a small amount of cytoplasm as a cap on one of the nuclear poles, is regarded as the youngest form of neuroblast. By the development of two processes centrally and peripherally, the bipolar neuroblast is produced. The proximal process atrophies and the cell assumes a pear shape with a long process from the narrowest end, giving rise to the picture of the unipolar neuroblast. Its cone of growth has proliferated so that it already lies out in the mesoderm. Shortly after this transformation the nucleus swells, 1—2 nucleoli are produced, and the multipolar neuroblast is formed, with numerous processes. The multipolar neuroblast passes into the complete nerve cell, which process is considered to be terminated when accumulations of Nissl's bodies in the cytoplasm can be brought out with basic dyes. Between these various stages HIS believed himself to have found transitional forms. The neurofibrils can be shown at an early stage in the neuroblasts, in chick embryos from the third day of incubation and even earlier (HELD 1898, CAJAL 1899, GONZALES 1938).

Cytological investigations with ordinary staining methods are of more immediate interest for comparison with cytochemical data. SCOTT (1899) investigated the development of nerve cells in embryos from different mammals after staining with toluidine

blue-erythrosin. In the embryonal cells supposed to be transitional between the germinal cell and the apolar neuroblast, SCOTT observed a disappearance of the basophil substance in the nucleus, but an increase of the acidophil. Synchronous with this nuclear change, a substance with a marked affinity for toluidine blue appeared in the cytoplasm closely round the nucleus. In further developed neuroblasts SCOTT found that the eosinophil substance in the nucleus showed an increase, as also the basophil substance in the cytoplasm, whereas the basophil substance in the nucleus continued to diminish. In SCOTT's opinion these pictures showed that the chromatin of the nucleus directly participated in the formation of Nissl's bodies in the cytoplasm. According to SCOTT, the primitive cell between the germinal cell and the apolar neuroblast corresponded to His' transitional cell with developed nucleus and richly stainable outer cap of cytoplasm. SCOTT considered, however, that this last-mentioned cell represented a later stage of development than the primitive neuroblast, as the basophil substance in this cell had not yet diffused into the cytoplasm. According to SCOTT, the basophil substance in the cytoplasm is not distinctly observable until a considerable lapse of time after the complete development of the neuroblast and its migration into the mantle layer.

HATAI (1904) examined rat embryos 10—13 mm in length with the same technique. The nuclei of most of the ganglion cells were situated peripherally. On staining with toluidine blue-erythrosin HATAI found that the nuclear membrane towards the centre of the cell was folded. In the nucleus close to this area he observed accumulations of particles stainable both with toluidine blue and with erythrosin, which were not present close to the non-folded parts of the nuclear membrane. Nissl's bodies were first shown in the cytoplasm in the vicinity of this irregular part of the nucleus. As soon as these irregularities within the part of the nuclear membrane facing the centre could no longer be shown, also other changes could be observed in the ganglion cell. The above-mentioned acidophil and basophil granules near the nucleus could not be demonstrated. The content of acidophil granules in the nucleus had increased. The cytoplasm was filled with Nissl's granules. In HATAI's view these pictures showed that Nissl's bodies were formed by the migration of chromatin substance out from the nucleus. This view was shared by SCOTT (1898), HOLMGREN (1898) and ROHDE (1903).

COLLIN (1907) states that, when the tigroid bodies begin to be differentiated in the neuroblast, the nuclear chromatin decreases.

RIESE (1939), in experiments on bears, investigated the development of the nerve cell in the occipital lobe. The author attached the greatest importance to the distribution of the basophil substance in the neuroblast at different stages of development. According as the basophil substance increased in the cytoplasm, he found that the nucleus paled.

For this investigation it is essential also to know the rate of growth and the relative size during the different stages in the development of the nerve cell.

The nerve cell undergoes its last division at an early stage of the embryo life. MORPURGO and TIRELLI (1892) showed that the cell-divisions in a spinal ganglion of a rabbit embryo ceased at a length of 4 cm.

KAISER (1891) took measurements of the anterior horn cells in human embryos and recorded a growth from  $700 \mu^3$  at the end of the first month to  $124700 \mu^3$  at birth. BOMBICCI (1899) found that the neuroblast in chicks showed but little growth during its migration into the mantle zone. Not till the sixth or seventh day of incubation did its volume begin to increase.

BUSACCA (1920, 1923) found that, as compared with the body-weight, the growth of the ganglion cell proceeded more slowly both absolutely and in percentage. LEVI (1907) believed himself to have established that the nerve-cell volume was a function of the body surface. Whilst the body mass increased approximately in proportion to the cube of the body-length, the nerve cell increased with the square of the latter. According to LEVI, BUSACCA, HEIDENHAIN and others, the nucleus-plasma ratio decreases during the development of the nerve cell. This decrease is more marked in the larger cells than in the smaller.

DONALDSON and NAGASAKA (1918) showed that so long as the body surface increased, the ganglion cell volume increased in proportion.

Data regarding the growth of the neurite are scanty. DONALDSON and NAGASAKA (1918) determined the increase of calibre in neurites of anterior horn cells and spinal ganglion cells in rats from 17 to 360 days after birth. They found that the diameter of the nerve fibres increased more rapidly than the ganglion cell. This applied particularly to the motor nerve fibres. The growth of the neurite was proportional to the increase in body surface.

AGDUHR (1920) found that the cross-sectional area of the coarsest spinal-nerve fibres in dogs was decoupled from the sixth day after birth to the sixth year. The increase in calibre, however, varied markedly in the different roots, namely between 4 and 12 times. We must also consider the peripheral branches of the neurone, in regard to which exact data are naturally unavailable. According to TELLO (1917), the intramuscular plexus branches off successively as new muscle fibres are formed. In chicks after the 15th day of incubation, when the author states that the number of muscle fibres is fixed, one can merely observe a thickening of the neurites, myelinization and the development of nerve-endings.

The volume of the neurone is bound to be considerably increased also by the development of dendrites, which takes place at an early stage.

The most thorough investigations of the rate of growth during the development of the nerve cells have been made by LEVI and collaborators (1897—1925). LEVI showed that the growth in the nerve cell-elements at an early stage of development was but little marked. *Not until the neuroblast acquires the cytological characteristics of the young nerve cell does the growth proceed at a rapid rate, which is maintained during the whole intrauterine life.* Postnatally it immediately slows down very considerably. BU-SACCA (1916) showed that the volume of the ganglion cells increased during the whole period of bodily growth, and that this increase in volume was most marked during the embryonal period and diminished progressively after birth. OLIVO, PORTA and BARBERIS (1932), as also RONDININI (1936), investigated the growth of spinal ganglion cells in chicks and mice during the embryonal period as well as postnatally, and found that the rate of growth of the ganglion cells during the embryonal life was very high from the sixth day of incubation and the eleventh fetal day, respectively, and decreased on hatching, first rapidly and then slowly. They found that the rate of growth of the neuron was directly proportional to the body weight. The rate was higher for the ganglion cells with a larger cell volume, but from the thirtieth day after birth it was the same for all categories of cells. PILATI (1938) obtained similar results in examining the spinal ganglion cells in man. On an average the cells increased from the 59th fetal day to birth from  $327 \mu^3$  to  $1900 \mu^3$ . When the ganglion cells were divided into different size-groups, it was found that the smallest cells had increased from  $65 \mu^3$  to  $697 \mu^3$ , and the largest

from  $1767 \mu^3$  to  $61601 \mu^3$ . From the 70th embryonal day a marked reduction in the rate of growth could be observed. Between the 59th and 70th day the smallest cells grew more slowly than the other groups. From the 70th day to partus the rate of growth of the medium-sized ganglion cells was greater than that of the largest and smallest cells. Post partum the growth of the largest cells proceeded more rapidly than that of the others. PILATI observed a steady growth during the entire life of all the ganglion-cells in man and a more marked growth up to the 29th year.

## B. Own Investigations.

### 1. Some Definitions.

The cytological definitions of certain cell organelles are very fluid. As some of them can be shown to be integral parts of the protein-forming system in the cell, and on this basis can be cytochemically defined with greater exactness than general cytologic procedure permits, the following terminology has been used in the sequel.

The term *nucleolus* is cytologically by no means easily definable, and has been used for various constituent parts of the cell-nucleus. In the previously cited works (CASPERSSON, the author and collaborators) it has been shown that at any rate the bulk of the nucleolar substance has developed from chromocentre-like nuclear parts, probably derived from heterochromatin, and are connected with the cytoplasmic protein-forming mechanism. In the course of the present investigations it has been found possible to tackle this problem in greater detail and to adduce further evidence in support of this view. It accordingly seems justified to define the term "nucleolus" more precisely as follows: *a structureless body, rich in hexone bases, and containing varying amounts of ribose nucleotides, but no demonstrable ribodesose nucleic acid, apart from isolated small particles which may be interspersed.* The nucleolus is often surrounded by a wreath of granules rich in ribodesose nucleotides. This terminology has been adopted throughout in the sequel.

*Chromocentre* should be understood to mean *a collection of particles rich in ribodesose nucleotides, concentrated in a ground-substance, which, on analysis with ultraviolet ray absorption measurements and a determination of the capacity for binding acid*

dye groups, can be shown to have the same essential composition as the nucleolus, that is, *proteins rich in hexone bases in a very highly concentrated form as well as ribose nucleotides.*

*Heterochromatin.* Apart from particularly suitable cytologic material from invertebrates, heterochromatin has been very badly defined. Also from a cytogenetic point of view, it can be defined only under specially favourable conditions. Investigations with ultraviolet ray absorption methods on particularly favourable material have shown that the chromocentre substance which, as stated above, is associated with the development of the bulk of the nuclear substance, is derived from parts which are certainly heterochromatic. As ultraviolet analysis makes it possible fairly well to define the bodies in question, even in material which cytologically and, with the present cytogenetic methods, is so unfruitful as, for example, the mammalian cells here examined, *heterochromatin in the interphase nucleus* may be defined as *largish, distinct chromocentres in close association with the nucleolar apparatus.* To what extent this cytochemical definition will cover the cytological and cytogenetic, can be determined only by new and more delicate procedures within these two last-mentioned spheres.

## 2. Analyses of Neuroblasts with Cytochemical Methods.

The material examined consisted of altogether 99 embryos:

<i>human embryos</i>	<i>rat embryos</i>	<i>rabbit embryos</i>
35 mm (2)	3 mm (2)	20 mm (9)
60 mm (3)	5 mm (4)	25 mm (3)
150 mm (2)	10 mm (9)	
	11 mm (12)	
	12 mm (14)	
	13 mm (12)	
	15 mm (7)	
	20 mm (4)	
	23 mm (10)	
	25 mm (3)	
	36 mm (3)	

Fixation: Gersh's freezing-drying method and Carnoy's solution. In the examination of this material the following technique

was employed. The material was sliced in serial sections 5  $\mu$  in thickness. Section 1 was stained with toluidine blue-erythrosin. Section 2 was used for photographing in ultraviolet and for the selection of suitable objects for measurement. On section 3 the Feulgen reaction was performed. Section 4 was impregnated with silver in accordance with Bodian's method for the control of the neuroblasts. Section 5, again, was used for staining with toluidine blue-erythrosin, and so on.

In order to show the differences in the amount of proteins in the embryonal nerve cell at different stages of development, the cell volume in the various cases was computed under the assumption that the cells were prolonged rotation ellipsoids. As axis values the author took the largest and smallest cell diameters in a section of the cell containing the major part of the nucleolus and consequently passing through the centre of gravity of the cell, or at any rate near that point. It was also essential that the section should contain cytoplasm at the base of the neurite, which afforded a guarantee that the large axis would correspond with the maximum diameter of the cell. In computing the total amount of proteins one must take into account not only possible errors in the actual computation of volume, but also those which may be due to the treatment of the material with fixatives and other liquids used in the histological technic, as well as variations in the thickness of the sections. The shrinkage resulting from treatment in accordance with the usual histological technic, may be estimated at about 20 per cent (PATTEN and PHILPOTT 1921, HERTWIG 1931), being fairly uniform in similarly treated material. Though it is difficult to make an exact estimate, the errors resulting from these sources should, taken separately, not exceed some 10 per cent in the determination of the proteins, except in particularly complicated cases. Seeing that the differences in the cells at the different stages of development, as shown below, were about ten times as large, the errors are of subsidiary importance for the demonstration, in principle, of the protein changes. As the cytochemical investigations of all the 99 embryos corresponded very well at the different stages, only three series will be adduced below as *typical examples*. In these series thorough ultraviolet absorption measurements were also taken, in each case four absorption series. In order to obtain data independent of the ultraviolet analyses in regard to the basicity of the proteins, all the sections were stained, including those in which ultraviolet

measurements were not taken with acid dyes in accordance with the above indicated method.

The results obtained in the course of the investigation in regard to unipolar and multipolar neuroblasts from rat embryos 10—13 mm in length are set forth below as typical examples. These two stages in the development of the nerve cell are in fact found simultaneously well-developed in rat embryos of that size, and can therefore be analyzed in one identical section. In such comparisons the source of error arising from variations in the thickness of the sections will naturally be eliminated, and the shrinkage may be assumed to be uniform in both cases. A typical example of young nerve cells, in a 23 mm rat embryo, is also adduced.

a. *Analyses of unipolar neuroblasts.* The unipolar neuroblast had an elongated nucleus of elliptical shape as well as a cytoplasm showing intense absorption at a wave-length of 2570 Å, situated like a cap on the nuclear pole facing the marginal layer (see Fig. 4). Round the remaining part of the nucleus the cytoplasm mass was very poorly developed. The volume of the cells measured was on an average  $75 \mu^3$ , that of the nuclei  $50 \mu^3$ . The volume of the nucleus relatively to the cytoplasm was on an average in the ratio of 1.97 : 1. With the aid of Bodian's method of impregnation the neurofibrils in the growing neurite were brought out. The photograms in ultraviolet showed that the neuroblast nucleus had a larger content than the adult nerve cell of particles containing nucleic acid. The Feulgen reaction showed that the nucleic acid in these particles was ribodesose. No nucleolus containing ribose nucleic acid was found in these cells. The nuclear structures which, on staining with toluidine blue-erythrosin, resembled the nucleolus described by other authors, contained mainly ribodesose nucleotides, being thus comparable with a chromocentre area.

Curves 1 and 2 in Fig. 5 show an example of absorption spectra taken at a point in the cytoplasm of neuroblasts at this stage of development. At a wave-length of 2600 Å a distinct absorption maximum appears, indicating the occurrence of large concentrations of nucleic acid. At 2800 Å a protein band becomes visible, as is clearly shown by the bend in the profile of the curve. The tyrosin maximum is found at a wave-length of about 2850 Å. Also the capacity for binding acid dye-groups is in general far higher than in the multipolar neuroblasts. From this

it is evident that proteins rich in hexone bases are contained in the cell substance. The other cells measured yielded curves of a similar shape. The quotient  $\varepsilon_{260}/\varepsilon_{280}$  varied from 1.56 to 1.70 in different cells. The quotients are computed with due allowance for the light scattering in the preparation. If we adopt a "standard protein" with an average content of 5 % tyrosin and 1.5 % tryptophan (see p. 16) and compute on the basis of this quotient the ratio between the content of proteins and nucleic acid, the result obtained is that the protein concentration stands to the

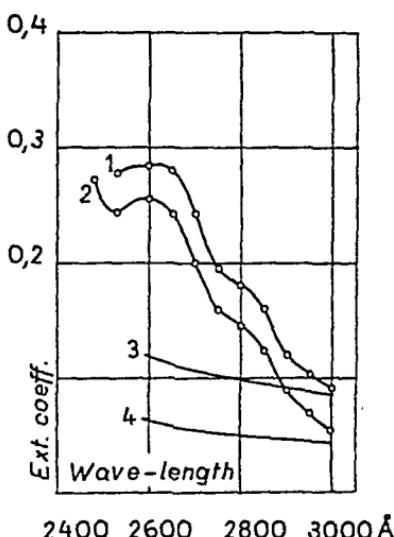


Fig. 5. Curves 1 and 2 from points in the scantily developed cytoplasm in unipolar neuroblasts. Curves 3 and 4 show the estimated loss of light by scattering. (See p. 18.)

nucleic acid concentration in the ratio of 2 to 6 : 1. Micro-incineration of the nearest-lying section showed, at the site of the cytoplasm intensely absorbing at 2600 Å, a substantial residue of ash. In the adult nerve cell the axon hillock contains but a small amount of nucleotides as compared with the remainder of the cytoplasm. In these neuroblasts, on the other hand, the place from which the neurite issues is marked by the same high concentration of nucleotides as the cytoplasm in general.

b. *Analyses of multipolar neuroblasts.* Nearer the marginal layer in the neural tube in rat embryos 10—13 mm in length lie somewhat larger neuroblasts with a different cytological picture (Fig. 6). Some of them are multipolar neuroblasts. The nucleus in most cases is of spherical shape, and the cell has a more rounded form than the unipolar neuroblast. The cytoplasm surrounds the entire

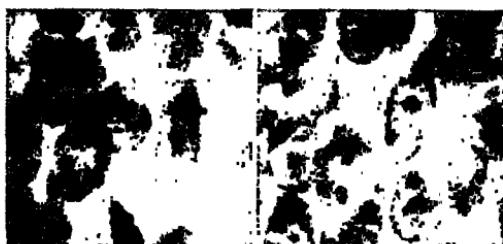


Fig. 4. A group of unipolar neuroblasts with a cap of intensely absorbing cytoplasm. Carnoy-fixed. Magnification  $1,150 \times$ . Objective aperture 0.85. Condenser aperture 0.6.

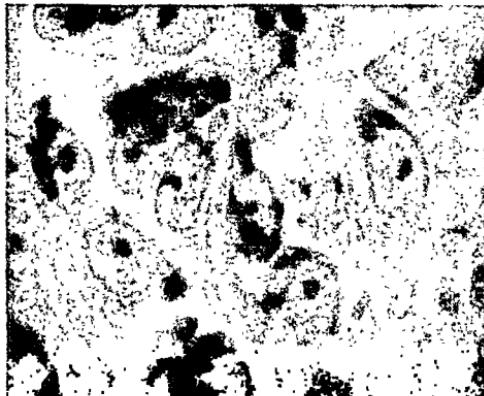
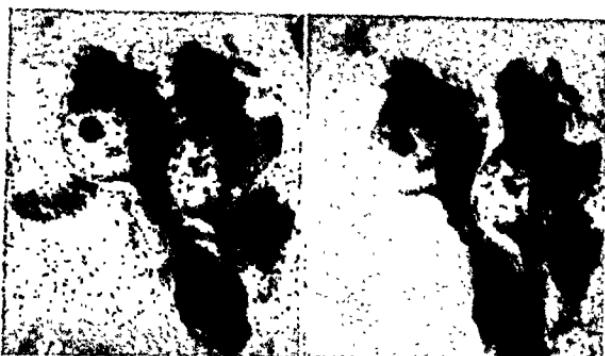


Fig. 6. A group of multipolar neuroblasts from the same section as in Fig. 4. Magnification and optics as in Fig. 4.



a. b.

Fig. 8 a. Young anterior horn cell from a 23 mm rat embryo. Fig. 8 b. The same cell, but dissected for ultraviolet absorption measurement. Carnoy-fixed. Magnification 1,150  $\times$ . Objective aperture 0.85. Condenser aperture 0.6.



Fig. 9. Embryonal spinal ganglion cells from a 25 mm rabbit embryo. The nuclear membrane area towards the centre of the cell flattened, folded and rich in nucleotides. Carnoy-fixed. Magnification 1,150  $\times$ . Objective aperture 0.85. Condenser aperture 0.6.

nucleus and is more developed than in the unipolar neuroblast. It is characterized by a considerable absorption at 2570 Å. The cells measured had an average volume of  $204 \mu^3$ . The volume of the nucleus amounted to  $74 \mu^3$ . The volume of the nucleus relatively

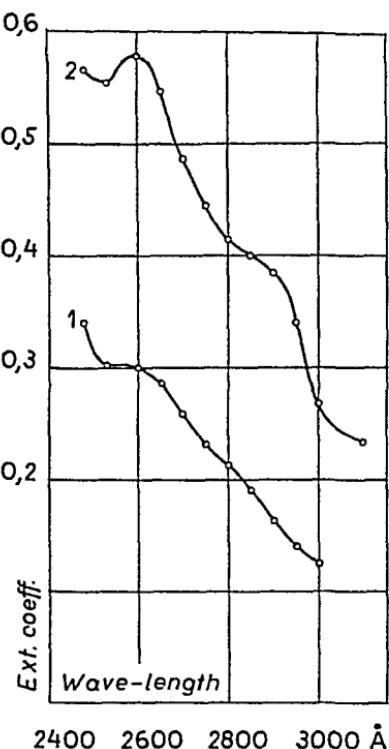


Fig. 7. Absorption spectra from a point in the cytoplasm of a multipolar neuroblast (curve 1) and a young anterior horn cell (curve 2).

to that of the cytoplasm in the average cell was thus in the ratio of 1:1.7. The photograms in ultraviolet at the maximum absorption of nucleic acid showed that the nucleus relatively to its volume contained a smaller number of particles rich in nucleotides than the unipolar neuroblasts. In the large nucleus one or two largish Feulgen-positive particles appear, which chromocentre cytologically resembles nucleoli. In a number of cells the nuclear membrane is folded in certain sections and rich in nucleic acid. Fig. 7 curve 1 shows an example of an absorption spectrum at a point in such a cell lying about  $100 \mu$  from the above-mentioned young neuroblast. The curves show a nucleic acid band at 2600 Å. An analysis shows also a protein component. The protein absorption maximum is poor compared with that of the nucleotides. The tyrosin maximum lies between 2800 and 2850 Å. The shape of

the curve indicates a type of absorption intermediate between the typical absorption spectrum for proteins rich in hexone bases and proteins of the globulin type. In regard to the capacity for binding acid dye groups see the above remarks. The other cells yielded curves of the same shape. The quotient  $\epsilon_{260}/\epsilon_{280}$  varied from 1.4 to 1.57 and was computed with due allowance for the scattering of light in the preparation. Reckoned as "standard protein" (see p. 16), this signifies that the protein concentration stands to the nucleic acid concentration as 6 to 13 : 1.

Micro-incineration of the multipolar neuroblast yields, as in the case of the unipolar neuroblast, a substantial residue of ash at the site of the cytoplasm intensely absorbing at 2600 Å.

### 3. Analyses of Young Nerve Cells.

In rat embryos 23 mm in length the anterior horn cells have a very characteristic appearance (Fig. 8 a and b). The nucleus has a spherical shape and is situated eccentrically. The cytoplasm is well developed and absorbs very intensely, compared with that of the neuroblasts at 2570 Å. The cells measured had an average volume of  $1065 \mu^3$ . The nuclear volume was  $382 \mu^3$ . The volume of the nucleus relatively to that of the cytoplasm in the average cell is in the ratio of 1 : 2.8. Relatively to its volume, the nucleus has a very small content of particles rich in ribodesose nucleotides. In its centre a rounded structure (in certain cells two) containing large concentrations of nucleotides can be observed. As the Feulgen test for this structure was entirely negative, the nucleotides must be of ribose type. In staining tests these structures took up acid dyes with great avidity. They are thus nucleoli. Surrounding the cell organelles rich in ribose nucleotides, a thin peripheral layer of particles rich in ribodesose nucleotides can be observed. This cell substance rich in ribose nucleotides may be designated as a nucleolus, in contradistinction from the chromocentre-like formations in the neuroblasts, as this has been found to be the characteristic composition of the large nucleoli, rich in ribose nucleotides, in the adult nerve cells. This observation will be discussed in greater detail in the sequel. (See Chapter I Part II.)

Fig. 7 curve 2 shows an absorption spectrum measured in a section of the cytoplasm which is dark in the ultraviolet picture.

At 2600 Å a marked nucleic acid band is observed. At 2900 Å a distinct absorption band appears, which then rapidly fades as the wave-length increases. The tyrosin maximum lies near 2950 Å. Acid dye groups are absorbed with an avidity which is even greater than in the case of the unipolar neuroblast. The cytoplasm substance thus has a high content of proteins rich in hexone bases as well as large concentrations of nucleotides. As the latter give a negative result to the Feulgen test, the nucleotides must be of ribose type. The quotient  $\epsilon_{260}/\epsilon_{280}$  varied in the measured cells from 1.4 to 1.5, and was computed with due allowance for the light scattering in the preparation. Reckoned as the above-mentioned "standard protein", this signifies that the protein concentration stands to the nucleic acid concentration in the ratio of 8—13 to 1. The ribose nucleotides in the cytoplasm are accumulated in certain limited parts, which on subsequent staining of the same section with a basic dye were found to agree well with the cytological picture of Nissl's bodies.

On micro-incineration both the nucleolus and the accumulations in the cytoplasm which absorb intensely at 2600 Å yielded a substantial residue of ash.

#### 4. Nucleolar Apparatus and Protein Increase. A Survey.

##### a. Development of the Nucleolar Apparatus.

The development from unipolar neuroblast to young nerve cell was followed with different cytochemical procedures. The results obtained with material from rat embryos 10—13 and 23 mm in length have been adduced as typical examples. In these investigations it was found possible to follow the development of the nucleolus and of the large amounts of cytoplasmic nucleotides.

The unipolar neuroblasts have an average volume of  $75 \mu^3$ . The cytoplasm is small in proportion to the nucleus. The ratio between the volume of the nucleus to that of the cytoplasm may be estimated as 2.0 to 1. An accumulation of ribodesose particles, which may be said to constitute a chromocentre area, is seen in the dominating nucleus. The small amount of cytoplasm accumulated close to the pole of the elongated cell from which the neurite issues contains rather large concentrations of ribose nucleotides. The protein concentration stands to the nucleic acid concentration as 2—6 to 1.

The multipolar neuroblasts measured have on an average a volume of  $204 \mu^3$ . The cytoplasm in this type of cell is more developed. The ratio between the volume of the nucleus to that of the cytoplasm was estimated as 1 to 1.7. The nucleus is on an average larger than in the unipolar neuroblast and contains distinct chromocentre areas with a ribodesose nucleotide content, which, on examination with ordinary cytological staining methods, resemble nucleoli. The nuclear membrane in some cells within certain areas is folded and rich in nucleic acid. The cytoplasm contains ribose nucleotides in similar concentrations as in the unipolar neuroblast. The protein concentration stands to the nuclein acid concentration as 6—13 to 1. Absorption spectra show that the amounts of absorbing substance are comparable with that of the unipolar neuroblast, which indicates that the amount of protein has increased concurrently with the increase in the volume of the cell.

The young nerve cells analyzed had an average volume of  $1065 \mu^3$ . The ratio between the volume of the nucleus to that of the cytoplasm was estimated as 1 to 2.8. The nucleus is large and spherical and contains in proportion to its volume but a small amount of ribodesose particles. In its centre one observes a large nucleolus, rich in ribose nucleic acid, on the periphery of which one sees a fine network of Feulgen-positive elements. On the ultraviolet photograms the nuclear membrane is shown to be rich in nucleic acid and in certain sections folded. The well-developed cytoplasm contains ribose nucleotides in large concentrations accumulated in certain parts of the cytoplasm, which on subsequent staining with a basic dye corresponded well with the cytologic picture of Nissl's bodies. The absorption measurements and dye-binding tests show that the content of proteins, rich in hexone bases, in the cytoplasm is particularly large. The proportion between the proteins and the nucleotides is approximately the same as in the multipolar neuroblast.

Both nucleus and cytoplasm increase in volume during the development of the neuroblast into a nerve cell, and the largest increase is shown by the cytoplasm; the "nucleus-plasma relation" is thus shifted in favour of the cytoplasm, as had already been pointed out by HEIDENHAIN in 1911. An important fact can thus be noted, namely that *the large amounts of ribose nucleotides in the growing cytoplasm do not appear until after the development of the nucleolus rich in ribose nucleic acid.*

Earlier cytochemical observations of rapidly growing embryonal cells from chicks (CASPERSSON and THORELL 1941) have served as a basis for dividing the development of such cells into three stages with a different mitosis frequency. Stage I is an early embryonal phase with very intensive growth. The cell nuclei include chromocentre areas with parts containing both ribodesose and ribose nucleotides. Stage II is a later embryonal phase with a reduced intensity of growth. These cells have a well-developed nucleolar apparatus rich in ribose nucleotides and a cytoplasm of larger volume, likewise containing ribose nucleotides. Stage III marks a transition to an adult stage with the cessation of growth. In these cells the nucleoli are reduced in size and the cytoplasm is poor in nucleic acid.

Stages I and II are well covered by the development of the unipolar and multipolar neuroblasts. Afterwards the nerve cell shows a development deviating from that of the average embryonal cell (stage III). In the young anterior horn cell the nucleus and especially the cytoplasm undergo an immense increase in volume. During this time the nucleolus becomes continuously larger and is charged with ribose nucleic acid, after which increasingly higher concentrations of ribose nucleotides are observed in the cytoplasm. This phase thus differs very considerably from the corresponding stage III in the other above-mentioned somatic cells. Thus, *instead of the cessation of the growth, with a reduction in the size of the nucleolar apparatus, in stage III, the nerve cell passes through a period during which the nucleolar material as well as the cytoplasm nucleotides rapidly increase, in order to remain well-developed during adult life.* According to the views set forth above, this should signify an intensive production of cytoplasmic proteins even during that period.

Here we find an interesting parallel with the oocyte, in which, as is well known, there is a second period of growth with a very rapid development of the cell. This seems to correspond to stage III in the development of the nerve cell, which may be designated with good reason as a "second period of growth" in that cell.

#### b. Increase in the Amount of Protein.

From the growth of the cell, i. e. the increase of the cell volume, an augmentation of the proteins in the cell cannot, of course, immediately be inferred. Such an increase can, however, be

estimated from the results of the ultraviolet absorption measurements.

The increase in the cytoplasm of the nerve cell in stage III is very considerable, and, if the volume of the neurite is included, enormous. An estimate of the volume of the neurite must obviously be very rough. For neurites in later stages of development the diameter, apart from the myelin sheath, was taken as a basis of measurement. The growth cone of the unipolar neuroblast lies out in the mesoderm (HELD 1909). In order to compute the thickness of the neurites, a number of them, taken from unipolar neuroblasts, were measured in sections impregnated with silver. The values were about  $1\text{ }\mu$ . Reckoned according to the mesoderm, the length may be estimated at  $50\text{ }\mu$ . Reckoning with the values of the measured cells, the volume of the cytoplasm plus that of the neurite will be  $100\text{ }\mu^3$ . For the multipolar neuroblast the diameter of the neurite was reckoned in the same way at  $1.5\mu$  and the length was estimated at 3 mm. The volume of the cytoplasm plus that of the neurite will then be  $7000\text{ }\mu^3$ . For the young anterior horn cell the diameter of the neurite was reckoned in the corresponding manner at  $3\text{ }\mu$  and the length was estimated at 15 mm, in the 23 mm rat embryo used for the measurements. The volume of the cytoplasm plus that of the neurite will then be  $142000\text{ }\mu^3$ . As a basis of comparison we may take an adult anterior horn cell with a mean diameter of  $50\text{ }\mu$  and a nuclear diameter of  $18\text{ }\mu$ . According to measurements of the anterior roots in adult rats (DUNCAN 1934), the mean diameter of the coarse fibres is about  $16\text{ }\mu$ .<sup>1</sup> Exclusive of the myelin sheath, a mean diameter of  $8\text{ }\mu$  and a length of 8 cm have been taken, in this example of measurements on rats, as a basis of computation. The volume of the cytoplasm plus that of the neurite will then be  $4,060,000\text{ }\mu^3$ . The total volumes of the nerve cell at different stages of development will thus show the following ratios:

Unipolar neuroblast	Multipolar neuroblast	Young anterior horn cell	Adult anterior horn cell
1	70	1420	40600

The ratios of the cytoplasm volumes will be as follows:

1	5	27	2200
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An analysis of absorption spectra from points in the cytoplasm showed that in the unipolar neuroblasts the content of proteins

<sup>1</sup> HÄGGQVIST (1943) found that the diameter of the coarsest nerve fibres, apart from the myelin sheath, in the upper part of the sciatic nerve from rats amounted to  $9.5\text{ }\mu$ .

was about 30 per cent, and in adult anterior horn cells in rats (see Chapter I Part II) likewise 30 per cent.

*It appears from these computations that the total amount of proteins in the nerve cell cytoplasm, even if the neurite is not included, increases more than 2000 times during the development of the nerve cell from neuroblast to adult cell with completed growth.*

The protein concentrations in the neurite are not known, but it may be presumed that they are not appreciably less than those in the major part of the cytoplasm. It will be seen from the above table that the total increase in protein *per neuron* is singularly large during the later stages of development.

The results set forth show that, even if the neurite is set aside, the amount of cytoplasmic protein increases rapidly during the stages in question. If the neurite is included, this increase must be designated as immense compared with all other embryonal cells, except the oocyte. *The development of the nucleolar apparatus thus shows also during "the second period of growth" a marked increase of protein, which conduces to bear out the view that this apparatus plays an important part in the formation of cytoplasmic protein in general.* A more detailed investigation of the cytochemical mechanism will be reported in the following section.

## 5. Detailed Analysis of the Nucleolar Apparatus.

The changes in the content of nucleotides and proteins in nerve cells at different stages of development have been discussed in the preceding section. For the sake of perspicuity some observations on cytological details will be submitted below.

The cytological mechanism during intensive functioning of the protein-forming system has been examined in detail in adult spinal ganglion cells of *Lophius piscatorius* (HYDÉN 1943). On the basis of this material it could be shown that the nucleolus and the ribose nucleotides were active during the intensive functioning of the protein-forming system, the formation of proteins in the cell being aided by the direct participation of those nucleotides and of proteins rich in hexone bases (See Chapter I Part II p. 70 and for further particulars HYDÉN 1943). During the phase when this apparatus functioned most intensively, the content in the nucleolus of ribose nucleotides and proteins rich in hexone bases showed a considerable increase. The nucleus was displaced towards the periphery of the cell and the nuclear membrane was at the

same time folded within a local section of the nucleus. Outside this area there were large accumulations of ribose nucleotides ("nuclear-membrane nucleotides") and proteins rich in hexone bases. In order to determine whether the marked similarity between the spinal ganglion cells in *Lophius piscatorius* and the embryonal nerve cells at those stages of development when the production of protein proceeded most rapidly was due to the existence of the same mechanism in both cases, or was merely an apparent resemblance, a limited number of cells were examined with the above described cytochemical methods.

The material for examination was taken from rat and rabbit embryos 10—13 mm and 25—33 mm in length, respectively. The material was fixed in accordance with Gersh's freezing-drying method and in Carnoy's solution.

**Ultraviolet microscopy.** Fig. 9 shows a photograph of spinal ganglion cells from a rabbit embryo 25 mm in length, taken at 2570 Å. It is significant that most of the nuclei are excentrically situated in the cell, just as in the spinal ganglion cell of *Lophius piscatorius*. The nucleus is spherical. The nuclear membrane is irregularly folded and flattened towards the centre of the cell. In the nucleus one or two largish intensely absorbing particles and a number of smaller particles of that nature are observed. Examination showed that they consisted in one or two nucleoli surrounded by small groups of particles containing ribodesose nucleotides.

At 2570 Å the absorption in the cytoplasm is most marked in the peripheral parts and within the small area close to the folded section of the nuclear membrane. In a central part of the cytoplasm the absorption within one area at this wave-length is low.

**Absorption measurements.** Fig. 10 shows, by way of example, two absorption spectra from points in a cell of type just indicated. Curve 1 is taken at a point in the cytoplasm shown in the chart. At 2600 Å a distinct absorption maximum appears, which shows the presence of large amounts of nucleotides. At 2800 Å a marked protein band is seen. The tyrosin maximum lies at 2900 Å, which shows the occurrence of considerable amounts of proteins rich in hexone bases. Curve 2 is from a point in the lower absorbing cytoplasm area outside the folded part of the nuclear membrane. At 2600 Å a weakly marked nucleotide band appears. The tyrosin maximum lies at 2870 Å, indicating the basic character of the component protein. An analysis shows

the occurrence of very small amounts of nucleotides and large amounts of proteins rich in hexone bases. The central area in the cytoplasm has a far greater tendency to absorb acid dye groups than the peripheral. Investigations of the material from rat embryos gave the same results as those above described.

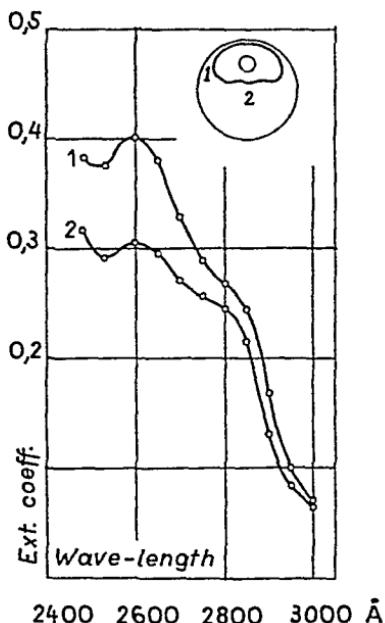


Fig. 10. Absorption spectra from points in an embryonic spinal ganglion cell, rabbit. The chart shows the measuring points.

A comparison between the distribution of proteins/nucleotides in the peripheral and the last-mentioned central part of the cytoplasm shows great differences. The quotient  $\varepsilon_{260}/\varepsilon_{280}$  was computed with due allowance for the light scattering and was for curve 1 = 1.43 and for curve 2 = 1.06. Reckoned as "standard protein" (see page 16), the protein concentration in the portion of the cytoplasm rich in nucleotides at the periphery will stand to the nucleic acid concentration as 10 : 1 and in the central cytoplasm mass outside the nuclear membrane as 60 : 1. In the cytoplasm outside the folded nuclear membrane rich in nucleotides, the nucleid acid component is thus very small relatively to the large concentrations of proteins. The results of the above reported detailed analyses of embryonal ganglion cells, in which there is a marked increase of cell protein, show a very good correspondence with the cytochemical picture in the spinal ganglion cells of *Lophius piscatorius*. From this it will be seen that the production

of the immense amounts of cell protein which takes place during the development of the nerve cell in mammals is effected by the same mechanism as in the ganglion cells of *Lophius piscatorius*, whence it would be justifiable to apply the observations made on these ganglion cells to mammalian cells.

## 6. The heterochromatin question in nerve cells from mammals.

The chromatin part which precedes the nucleolar apparatus in the protein-forming system in the cytoplasm, and which in certain invertebrates has been shown to coincide with the heterochromatin, may during the interphase be cytochemically defined as nuclear substance containing proteins rich in hexone bases as well as ribodesose and ribose nucleotides (CASPERSSON 1940, 1941). If the protein system functioned intensively in the nerve cell, the formation of distinct areas of this nature was to be expected. In order to investigate this question a series of experiments were conducted.

The cytological definitions of heterochromatin are so vague that in mammalian cells such regions can scarcely be identified with certainty. The possibility of attacking these questions cyto-genetically seems at present to be very small. The chromatin portion directly active in the protein-forming system can, on the other hand, be cytochemically well defined and corresponds in some invertebrate material with what can be shown there to be heterochromatin. The term heterochromatin has therefore been retained for that portion also in regard to mammalian material.

The material consisted of spinal ganglion cells, anterior horn cells in the spinal cord and Purkinje's cells from 10 rabbit embryos 22—33 mm. in length and 8 adult rabbits, half of them males, half of them females. Half of the material was fixed in accordance with Gersh's freezing-drying method and the rest in a 10 % formalin solution in saturated sublimate and Champy's solution. Altogether 14 absorption spectra in ultraviolet were taken on cells from different animals. As we are concerned here with a minute cell structure which it is difficult to analyze, the results of the investigations on the large adult spinal ganglion cells will be dealt with first.

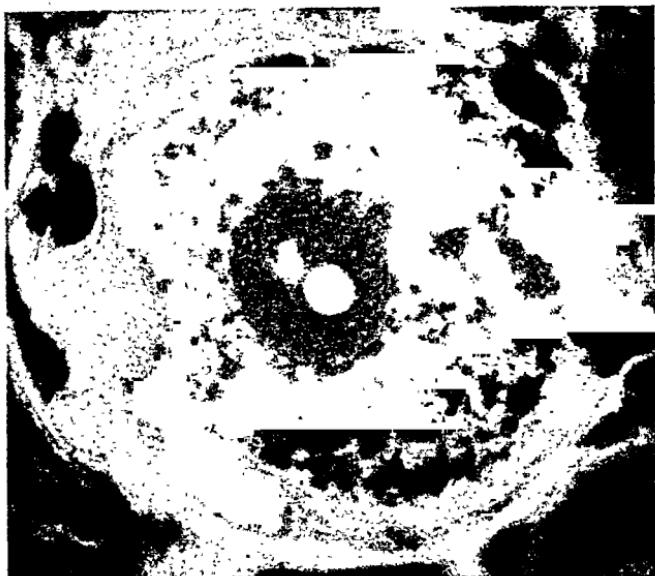


Fig. 11. Spinal ganglion cell, rabbit. Carnoy-fixed. In the nucleus, adjacent to the nucleotide-rich nucleolus, we see the chromocentre area, containing ribodesose and ribose nucleotide. Magnification  $1,150 \times$ . Objective aperture 0.85. Condenser aperture 0.6.

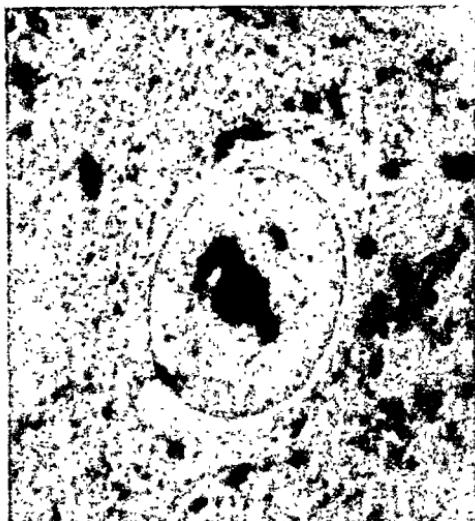


Fig. 14. Spinal ganglion cell, rabbit, stained with cyananthrol and photographed at  $5750 \text{ \AA}$ . The photograph indicates the great capacity of the nucleolus and chromocentre for binding acid dyes, as compared with that of the remaining nuclear substance. Magnification  $1,400 \times$ .

HYDÉN: Protein metabolism in the nerve cell.



### Spinal Ganglion Cells in Adult Rabbits.

**Ultraviolet microscopy.** In the nucleus, owing to the greater absorption in photographing at 2570 Å, certain parts stand out more than the nuclear substance in general. These cell structures are usually situated close to the nucleolus rich in ribose nucleotides or in its vicinity, and have the appearance indicated by Figure 11. Their size as a rule does not exceed that of the nucleolus. They are irregular in form. They vary in number in different cells. One or two large and several smaller absorbing areas of this nature are often found. In a few cells no such absorbing parts in the nuclear substance can be discovered. In some of these cells the nucleolus on the ultraviolet photograph is irregular, having one or more bulges. In others again there is only one absorbing cell structure of this nature lying close to the nucleolar substance.

**Absorption measurements.** Figures 12 and 13 show a series of absorption spectra taken at points in these absorbing areas close to the nucleolus (the curves marked 1) and at points in the

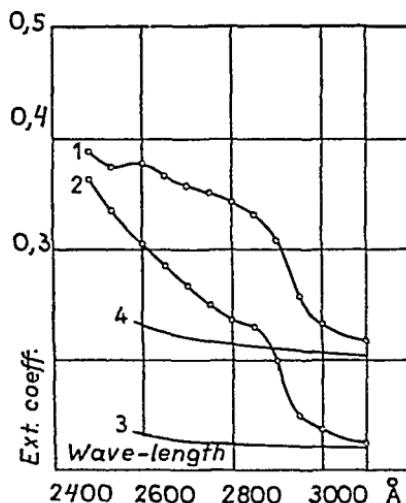


Fig. 12. Absorption spectra from the chromcentre in a spinal ganglion cell nucleus from a rabbit (curve 1) and from a point in the adjacent nuclear substance (curve 2). Curve 1 shows a nucleotide band and a marked protein band with a tyrosin maximum shifted towards long wave-lengths. Curve 2 shows merely a faint protein band. Curves 3 and 4 indicate the loss of light by scattering.

more weakly absorbing nuclear substance adjacent to it. (The curves marked 2). At 2600 Å a distinct absorption maximum appears in curve 1, showing the presence of nucleotides. At long

wave lengths we observe a marked absorption band, extending to 2950 Å. The course of the curve corresponds to a nucleic acid band overlapping a marked protein band, with its tyrosin maximum lying at long wave-lengths. The absorption of protein is high compared with the nucleic acid band and

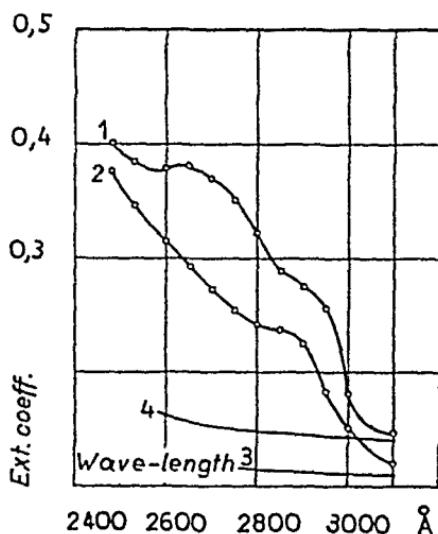


Fig. 13. Two absorption spectra similar to those in Fig. 12. Same notation.

gives the curves a characteristic appearance. The absorption spectra at points in the adjacent nuclear substance have merely a weak absorption maximum at 2850 Å, the tyrosin maximum lying at 2900 Å, which indicates the occurrence of small concentrations of protein with the absorption type of proteins rich in hexone bases. In regard to the capacity for absorbing acid dye groups see below.

*An analysis of these curves shows that the protein concentration in the absorbing substance close to the nucleus is three-times higher than the average of the adjacent nuclear substance, and that the nucleotide content is somewhat higher.*

**Feulgen reaction.** A comparison between the ultraviolet picture and the Feulgen picture showed that the substance analyzed here and defined as heterochromatin contains a larger amount of Feulgen-positive particles than the remaining nuclear substance. It is thus built up of a structureless mass of ribose nucleotides and proteins rich in hexone bases, whilst interspersed in it lie particles rich in ribodesose nucleotides.

**Micro-incineration.** In the micro-incineration of ganglion cells and photography in the dark field, large residues of ash were

obtained on the site of the nucleolus, as well as close to it within an area which in the ultraviolet picture corresponded to the above analyzed heterochromatic area, whereas the nuclear substance in general left but little ash.

#### Measurement of the Capacity for Binding Acid Dyes.

In staining spinal ganglion cells with acid dyes according to the technic described in Chapter 0, it is remarkable how intensively the nucleolus and the adjacent structure bind these dyes relatively to other cell structures (see Fig. 14). In measuring the absorptive capacity at the absorption maximum of cyananthrol at 5750 Å, the author obtained in some 10 measurements on an average the extinction coefficient 0.34 for the nucleolus, 0.28 for the absorbing substance close to the nucleolus and 0.09 for the remainder of the nuclear substance.

The results of the absorption measurements in ultraviolet point to the occurrence of proteins of hexone bases in the above analyzed structure close to the nucleolus. This method, however, gives mere indications of the amount of such proteins. Measurement of the capacity for absorbing acid dyes, on the other hand, gives, theoretically, a direct possibility of determining the amount of basic groups; and the relative contents of basic proteins can already be estimated from the preliminary measurements now made. They show plainly that *the nucleolus and the absorbing substance close to it contain very large amounts of basic protein groups as compared with the remainder of the nucleus substance.*

#### Anterior Horn Cells and Purkinje's Cells.

Investigations with ultraviolet microscopy, the Feulgen reaction, micro-incineration and staining with cyananthrol and ponceau under standard conditions were conducted on anterior horn cells and Purkinje's cells from the above specified rabbit material. About 40 series were arranged in this manner. The results of these investigations showed a similar distribution and composition of the chromocentre area in the three types of cells as was described above in regard to the large spinal ganglion cells of rabbits.

#### Embryonal Ganglion Cells.

In order to study more closely the formation of the heterochromatic chromocentre in adult ganglion cells during the develop-

ment of the nerve cell, a series of investigations were conducted in accordance with the same technic as for anterior horn cells and Purkinje's cells. About 30 series were arranged in accordance with these methods.

All these different methods gave similar results on cells at different stages of development from the rabbit embryos of varying size. The results of the investigation of nerve cells at later embryonal stages corresponded completely with those set forth above in regard to adult spinal ganglion cells, anterior horn cells and Purkinje's cells; they will therefore not be reported in detail.

At the earlier embryonal stages, namely unipolar and multipolar neuroblasts, there is as previously pointed out (see Chapter III p. 43) in the nucleus a well-developed area which is rich in particles containing ribodesose, and which does not differ from the corresponding structure in other somatic embryonal cells. According to the definition (see Chapter III p. 28), it may be designated as a chromocentre. This chromocentre is analogous in essentials with the above analyzed chromocentre in adult ganglion cells, but differs from it in detailed composition. The chromocentre in the earlier forms of neuroblasts includes a larger amount of particles rich in ribodesose nucleotides than the corresponding formation in the adult ganglion cells. A comparison between the ultraviolet picture and the Feulgen picture of this cell organelle in the embryonal and adult nerve cell shows that the difference is considerable.

In the unipolar and multipolar neuroblasts a nucleolus is missing. It was in fact not developed until the nerve cell passed through its second period of growths (termed above stage III) in connection with the formation of cytoplasmic nucleotides. It is first at this stage that one can show the heterochromatic chromocentre as it exists in the *adult ganglion cell*: *a group of particles rich in ribodesose nucleotides embedded in a ground substance of ribose nucleotides and proteins rich in hexone bases*.

The course of development of what has been here defined as a heterochromatic chromocentre thus corresponds well with the assumption that, as previously shown in regard to certain invertebrates, it is built up of a group of particles rich in ribodesose polynucleotides, which by forming proteins rich in hexone bases and ribose nucleotides, gives rise to the major part of the nucleolar material in the earliest phase of the cytoplasmic protein formation.

**Difference in the Heterochromatin in Males and Females.**

If the above analyzed nuclear structure in the ganglion cells of rabbit can be compared with heterochromatin in very intensive functioning, there is a theoretical possibility that disparities in its formation and frequency may be found in males and females, as the content of heterochromatin in the genome may be presumed to be considerably different in the two sexes.

The male rabbit in his chromosome set has an X and a Y chromosome. The female has XX. (BACHHUBER 1916, MASUI 1923.) The Y chromosome in many kinds of animals examined was largely heterochromatic, whereas the X chromosome usually shows this character only in part. The male rabbit will therefore presumably have a larger amount of heterochromatin in his chromosome set than the female, although the conditions in this regard have not yet been fully investigated. It should follow from this that in a type of cell such as the nerve cell, where the heterochromatin, in the capacity of regulator of the protein-formation in the cytoplasm, functions with special intensity, the size of the chromocentre areas will also reflect the differences in the heterochromatin content in the two sexes.

In order to settle this question, about 400 ganglion cells from rabbits were examined, half from males and half from females. Half the material was stained under standard conditions with cyananthrol, and half with ponceau. Only sections which included the major part of the nucleolus were examined

In the male rabbit the heterochromatic chromocentre could be defined in all the cells. In the female it was throughout more diffuse and not so well developed. In many cells (nearly half of the number of cases reckoned) in the female one sees merely irregularities in the general structure of the nucleus or in the vicinity of the nucleolus, which appear to correspond to this substance.

**Recapitulation.**

In ganglion cells from rabbits the author observed a nuclear structure containing ribodesose and ribose nucleotides as well as large concentrations of proteins rich in hexone bases. This structure thus corresponds to what was defined above (Chapter III,

p. 28) as a chromocentre. That organelle is situated close to the nucleolus. *During development it cannot be distinguished from the other nuclear substances until the time when the large nucleolus rich in nucleotides and the system of ribose nucleotides in the cytoplasm has been developed.*

This cell organelle corresponds well in structure and composition with the heterochromatic chromocentre in *Drosophila* (CASPERSSON 1940—41).

The composition of the nucleolus in *Drosophila* is characterized, in essentials, according to CASPERSSON, by a specially high content of proteins rich in hexone bases and ribose nucleotides. The same applies to the nerve cell nucleolus (see above p. 34 and Chapter I Part II p. 59). By measurements of the capacity for absorbing acid dye groups, the high content of basic proteins has been still more reliably determined.

*The heterochromatic chromocentre in Drosophila and the nuclear area in the ganglion cells of rabbits, as analyzed here, would thus seem to be identical structures.*

Furthermore the observations thus made strongly support the assumption, based on the *Drosophila* material, that the major part of the nucleolus material is formed in the heterochromatic nuclear section. The above reported studies of the nucleolar development are in good correspondence with such a mechanism.

The earlier cytological literature, for natural reasons, contains but scanty data regarding observations of structures in nerve cell nuclei which might be supposed to correspond to this analyzed chromocentre area. LEVI (1896) described acidophil bodies in the vicinity of the nucleolus in ganglion cells, and CAJAL (1911) states that, on impregnation, minute Ag-reducing particles in this nuclear area can often be observed. SAGUCHI (1930) describes weakly acidophil and basophil structures in ganglion cell nuclei, which in cytological character seem to resemble the chromocentre area analyzed here. SAGUCHI calls them nucleonephelium and considers that they migrate out into the cytoplasm, where they form a so-called cytonephelium, which in turn gives rise to Nissl's bodies.

## 7. Distribution of Nucleotides in Neural Tube and Spinal Cord.

The distribution in general of nucleotides within neural tubes and spinal cord also reflects the different intensity with which the endocellular processes of development, described in detail in the



Fig. 15. Outline view of Carnoy-fixed spinal cord rudiment from a 3 mm rat embryo. Note the nucleotide-rich zone close to the central canal. Magnification 200  $\times$ . Objective aperture 0.20. Condenser 0.20.



Fig. 16. Micro-incineration of spinal cord rudiment from a 5 mm rat embryo in the dark field. Corresponding to the parts absorbing at 2600, large amounts of ash. Magnification 40  $\times$ .



Fig. 17. Outline view of Carnoy-fixed spinal cord rudiment from a 23 mm rat embryo. In the anterior horns we see the young nerve cells with large aggregations of ribose nucleotides in the cytoplasm. They form dark patches in the anterior horn, otherwise poor in nucleotides. Magnification 200  $\times$ . Objective aperture 0.20.  
Condenser aperture 0.20.

preceding section, are carried on in different parts of the nervous system.

Fig. 15 shows an ultraviolet photograph of a section from a neural tube in a 3 mm rat embryo. The pillar zone close to the central canal contains chiefly bipolar and unipolar neuroblasts as well as vigorously growing spongioblasts with a large mitotic frequency. All these cells have a rather high content of ribose nucleotides in the scanty cytoplasm and a large amount of ribodesose particles in the nuclei, and all of them lie closely packed together. This accounts for the intensely dark appearance of the pillar zone in the ultraviolet picture.

Close to the marginal layer lie largish multipolar neuroblasts, whose nuclei contain a minor amount of ribodesose elements, and in whose cytoplasms the nucleotide concentration is still rather low. They have completed their last division, and increase in mass. In the ultraviolet picture they show as a light zone. Fig. 16 is a micro-incinerated section, photographed in the dark field, of a spinal cord rudiment from a rat embryo 5 mm in length. The residues of ash are largest in the pillar and mantle zones and correspond to the large amounts of nucleotides in those areas.

At a later stage of development sharply defined dark islands appear in the ultraviolet picture in the previously light zone close to the marginal layer. They consist of the young anterior horn cells, which during stage III have developed nucleotides in large concentrations in the growing cytoplasms. See Fig. 17, which is a spinal cord rudiment from a 23 mm rat embryo, photographed at 2570 Å. In the ultraviolet picture the dark band in the former pillar zone has almost completely disappeared.

At the adult stage the nucleotide islands at the anterior horns have increased in volume: in the ultraviolet picture they form large dark areas, whilst the previously dark zone in the area close to the central canal has completely vanished.

These changes in the distribution of nucleotides within the spinal cord rudiment reflect, broadly speaking, the intensity in the growth of the cells at the different stages of development. The occurrence and distribution of the two types of nucleotides furthermore correspond to the different periods of growth in the development of the nerve cell. In the zone close to the central canal the nucleotides consist particularly of ribodesose nucleotides in the nuclei, and assist in the development of the cell. In the

future anterior horns, on the other hand, the nucleotides consist mainly of the polyribose nucleotides lying in the nerve cell cytoplasm, which assist in the growth of the cell during stage III.

## 8. Survey of Results.

The investigations reported here, with the cytochemical methods described in Chapter II, are concerned with changes in the nucleotide and protein content of the nerve cell during the embryonal development, as well as with the cytochemical details during the course thereof.

During the embryonal development the nerve cell undergoes an immense increase in volume, to which there is no parallel in other somatic cells. Only the oocyte can show a similar increase.

*The unipolar neuroblast* has a poorly developed cytoplasm, containing ribose nucleotides and proteins in moderate concentrations. The nucleus is rich in granules containing ribodesose nucleotides. In the centre of the nucleus we observe a denser accumulation of elements rich in ribodesose nucleotides, forming a chromocentre. *The average multipolar neuroblast* has an about five times larger total amount of proteins in the cytoplasm. In the nucleus the above-mentioned chromocentre is left. Thus far the embryonal nerve cells correspond in development with other embryonal somatic cells. These latter cells, without any appreciable increase in substance, assume the character of adult cells, whilst the nucleolar apparatus and the cytoplasmic nucleotides are reduced in size (termed above stage III). *The embryonal nerve cell*, on the other hand, undergoes a very rapid growth during the corresponding stage III, whilst the total amount of proteins in the cytoplasm increases more than 2000 times over. During this period *a large nucleolus rich in ribose nucleotides appears, and a well-marked apparatus of ribose nucleotides develops in the cytoplasm.*

The chronological connection between the increase of proteins and the nucleolar development, which on this material could be followed in considerable detail, is indicative of a causal connection between those processes. In correspondence with previous investigations with similar methods on other cell material, the most probable explanation seems to be that *the development of the large nucleolus and the well-marked cytoplasmic nucleotide apparatus is the very mechanism which brings about the immense production of proteins.*

The cytochemical details in this "second period of growth" of the nerve cell were investigated on embryonal spinal ganglion cells. In these cells the nucleus is displaced towards the periphery of the cell, and that part of the nuclear membrane which is directed towards the centre of the cell is folded and rich in nucleotides. The cytoplasmic area lying outside this section of the nucleus contained ribose nucleotides and proteins rich in hexone bases in very large concentrations, compared with the peripheral parts of the cell. The function of the nucleolar apparatus had been previously investigated in detail by the author on the spinal ganglion cells of *Lophius piscatorius*, which have an intensively functioning cytoplasmic protein-producing system. In the phase where the functioning was most intense, the nucleus was displaced towards the periphery of the cell, the nucleolus was markedly enlarged in volume, the section of the nuclear membrane facing the centre of the cell was folded, and close to it ribose nucleotides in large concentrations were observed. The same cytochemical picture was shown in embryonal ganglion cells from mammals at those stages when a rapid increase of cell protein takes place. *This conduces to bear out the view that the marked increase of proteins during the embryonal development of the mammalian nerve cell is effected by a mechanism which is similar in all cells,* and that the different cytological pictures in the nerve cells in question merely represent stages of different intensity in the cytoplasmic protein-forming system, extreme cases being represented by certain spinal ganglion cells in *Lophius piscatorius*.

Prior to the nucleolus we find in the protein-forming system an earlier part, represented by the heterochromatin. In this case the term heterochromatin, also in mammals, is applied to the cytochemically well definable chromatin part primarily active in the cytoplasmic protein-forming system, as shown by CASPERSSON in regard to certain invertebrates. *In the above reported investigations it was possible to show regions in the nuclei of ganglion cells in rabbits, which are hypertrophied chromocentre areas, and which presumably correspond to the heterochromatic chromocentres in previously examined invertebrates.* (See p. 46.) They lie close to the nucleolus and contain nucleotide particles embedded in a ground-substance of ribose nucleotides and proteins rich in hexone bases in large concentrations. In embryonal ganglion cells areas with this structure can be first observed after the development of the large nucleolus and the cytoplasmic nucleotide apparatus.

### Summary, Chapter III.

The view that the formation of cytoplasmic proteins is induced by the production, from certain chromatin areas, of proteins rich in hexone bases, which form the major part of the nucleolus and afterwards at the nuclear membrane, with the aid of *ribose nucleic acids*, bring about the protein synthesis, has been based on collated observations of greatly varying cell material (see reference in Chapter II p. 10). In these investigations it has for the first time not only been *possible to deal thoroughly with all these processes on one identical cell material*, but also to follow them during *two* different protein-forming phases, namely an early embryonal development and the later development of the large protein masses of the definitive nerve cell. The results support in every respect the view just mentioned and extend it by observations of the detailed course of different processes. We are in short confronted here with nothing less than a universal cell mechanism.

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## P A R T I I.

# Nucleotide and Protein Metabolism in the Nerve Cell during Different Functional Conditions.

### Introduction.

As has been shown in the preceding section, the protein-forming system proceeds in the nerve cell, which does not deviate in this regard from the other somatic cells examined. The degree of development of the nucleolar apparatus may therefore be taken as an index of the intensity of the protein metabolism in the cell. As we are already so well acquainted with the detailed workings of this process in the nerve cell, scope is afforded for a finer analysis of that cell.

It being obviously out of the question to examine a nerve cell in complete rest, investigations have been made on young, well-fed animals at rest, and the results of these investigations have been compared with those on (1) animals in intense motor activity and (2) animals whose afferent nerves had been exposed to intense irritation.

The first step will thus be *to subject the cytochemical organization in different adult ganglion cells to thorough investigation.*

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## CHAPTER I.

### Adult Nerve Cells.

#### A. Literature.

According to the distribution of the basophil substance in the cytoplasm and the intensity of its staining, different authors have divided the various ganglion cells into several groups. NISSL (1895—96) divided the cells, broadly speaking, into somatochrome cells, with a markedly basophil and well-developed cytoplasm and karyochrome cells, which have a very small amount of cytoplasm and an intensely stainable nucleus. According to the degree of density in the stained parts of the cytoplasm, NISSL divided the somatochrome cells into pyknomorphous, apyknomorphous and parapyknomorphous cells. In the pyknomorphous cells the basophil parts were densely compressed. In the apyknomorphous the unstained parts of the cytoplasm took up much space. Finally, NISSL designated as parapyknomorphous those cells which in regard to capacity of staining were an intermediate form. In the literature there is a consensus of opinion that *nerve cells with large cytoplasm and long axons contain much basophil substance in the cytoplasm*. Basophil substance on the other hand is missing in karyochrome cells with a small cell-body and a short axon, such as the bipolar cells of the retina and the granular cells of the cerebellum.

CAJAL (1911) divided the nerve cells according to the appearance of the basophil substance into four groups. 1. Stichochrome cells with coarse granules of basophil substance. They included *inter alia* the motor horn cells. 2. Arkyochrome cells with a reticular arrangement of the basophil substance. Purkinje's cells *inter alia* were included in this group. 3. Gryochrome cells with the basophil substance in the form of granules. As a typical example CAJAL adduces the spinal ganglion cells. 4. Perichrome

cells with a basophil substance in the form of marginal granules. These include *inter alia* the molecular layer in the cerebellum.

FLESCH (1886—88) found, on the staining of nerve tissue with methylene blue, that even with a perfectly identical procedure some cells of the same type stained more intensively than others. He presumed that the chromophil and the chromophobe cells represented functionally quite different types of cells. NISSL (1894, 1895—96) considered that he could confirm this observation. In a later work (1896) he expressed the view that the picture of the chromophil cells had arisen *post mortem* in an artificial way by the technical treatment of the tissue, and that they were thus to be regarded as artefacts. EINARSSON (1933—35) studied the same problem with a method which he had elaborated for producing with gallocyanin-naphtazarin the basophil substance in the nerve-cell cytoplasm. He adheres to the view that the different capacity for staining is attributable to different functional conditions.

*Anterior horn cells.* The anterior horns contain two kinds of ganglion cells, namely root cells, the neurites of which project from the spinal cord with their anterior roots, and string cells, the neurites of which do not project from the spinal cord. In regard to the capacity for staining, the first-mentioned cells, according to the terminology used by NISSL, are somato-stichochrome. GOLDSCHEIDER and FLATAU (1898) described the occurrence of chromophil cells in the anterior horns and considered them to be artificial products. EINARSSON (1933) investigated, with his above-mentioned "specific staining method" for nerve cells, the cytology of the anterior horn cells. He describes pyknomorphous, para-pyknomorphous and distinctly chromophobe cells.

In the spherical, light nucleus there is a large nucleolus, which admits of being intensively stained with both basic and acid dyes. According to NISSL, two nucleoli may sometimes occur, the aggregate volume of which is equal to that of the solitary nucleolus.

*Spinal ganglion cells*, as previously pointed out, are examples of gryochrome cells with a basophil substance occurring in more or less fine granules. In dogs, according to LUGARO (1896), they can be divided into five different groups, according to the appearance of the basophil substance. The ones which most commonly occur are large, light cells, medium-sized, light cells, small, dark cells and small and medium-sized cells with marked granules. In very small number there occur large, light cells with con-

centrically arranged granules in the cytoplasm. VON LENHOSSEK (1897) describes, as the normal appearance of spinal ganglion cells in man, a finely granular form with a "Randschollenkranz", usually situated in the periphery, of more closely lying granules. As a deviation from this type, he regards a large, light cell form with a feebly stainable nucleus, a medium-sized form of cell with coarse granules and a small, dark cell form with a diffusely stainable nucleus. According to this author, the deviating cytological picture in this latter cell is due to greater density in the ground-substance of the protoplasm, and not to a larger amount of basophil substance. NISSL (1897) describes similar types of cell in the spinal ganglia of rabbits.

STÖHR (1923) states that in hematoxylin-eosin-dyed preparations smaller, darker and more homogeneously stained ganglion cells are observed, as compared with the larger types of spinal ganglion cells, which appear to be lighter and more finely granulated.

SAGUCHI (1930) distinguishes also between chromophobe and chromophil cells, stating that the latter are missing in rabbits.

KISS (1933) reports a procedure for obtaining in ganglia the cytological picture of chromophil cells, which he considers to be of sympathetic origin. FISCHER and RANSON (1933) sharply criticized the fixing method adopted by Kiss and showed the probable character of the results as artefacts.

EINARSSON (1933) divides the cells into a more darkly stained and a lighter type.

As appears from the above, a cytological observation made throughout in regard to spinal ganglion cells, is the occurrence, on staining with basic dyes, of larger, light cells and a smaller form, which admit of more intense staining with basic dyes both in nucleus and cytoplasm.

## B. Own Investigations.

### 1. Analyses of Anterior Horn Cells.

In order to obtain a survey of the composition of adult ganglion cells, cytochemical analyses, with the methods described in Chapter II, were made on cells from well-fed healthy animals at rest. The material of anterior horn cells was taken from rabbits

(9), cats (2) and rats (22). Fixation: Gersh's freezing-drying method and Carnoy's solution.

For the absorption measurements in ultraviolet, cells of *Nucleus myorhabdoticus*, and among them those within the anterolateral group, were selected. For the purpose of measurement, sections including the major part of the nucleolar substance were selected, for the following reasons. According to the investigation of LHERMITTE and KRAUS (1925), the anterior horn cells have an elongated form and have their largest axis in the longitudinal direction of the spinal cord. By selecting sections including the major part of the nuclear substance, which in undamaged cells is situated in the centre of the nucleus, results of the measurements in the nuclear substance from different cells can be compared in regard to the content of nucleotides and proteins.

Altogether 30 measuring series in ultraviolet were arranged. For the Feulgen reaction, in a number of cases, the same sections as for the absorption measurement were used. The cytochemical examinations of these anterior horn cells from the different animals corresponded very well, whence merely one serial investigation from the anterior horn cells of a rat need be adduced by way of example.

These results of the examination of anterior horn cells from different animals, with methods similar to those adopted here, have been reported in a previous work (LANDSTRÖM, CASPERSSON and WOHLFART 1941). It was established that the nucleolus contained large concentrations of ribose nucleotides, and in the cytoplasm there were likewise large concentrations of those nucleotides. On subsequent staining with basic dyes, it was found that the distribution of the aggregations of cytoplasmic nucleotides corresponded with the cytological picture of Nissl's bodies.

**Ultraviolet microscopy.** The cell adduced as a typical example is shown in Fig. 18. It was fixed in accordance with GERSH's freezing-drying method and was photographed at 2570 Å. The nucleolus shows an intense absorptive power, whereas the nuclear substance in general has a feeble absorbing capacity at this wave-length. The cytoplasm as a whole is marked by a very high absorptive power.

**Absorption measurements.** Fig. 19 shows a group of absorption spectra, and the chart indicates the measuring points in the cell.

**The nucleolus.** In the curve in Fig. 19, which has been taken

at a central point in the nucleolus, there appears a distinct absorption maximum at 2600 Å, indicating a high content of nucleotides. Above the nucleic acid curve proper we observe a protein band at 2800 Å, as a bulge in the profile of the curve. The tyrosin maximum lies near 2900 Å. Dye-binding tests showed

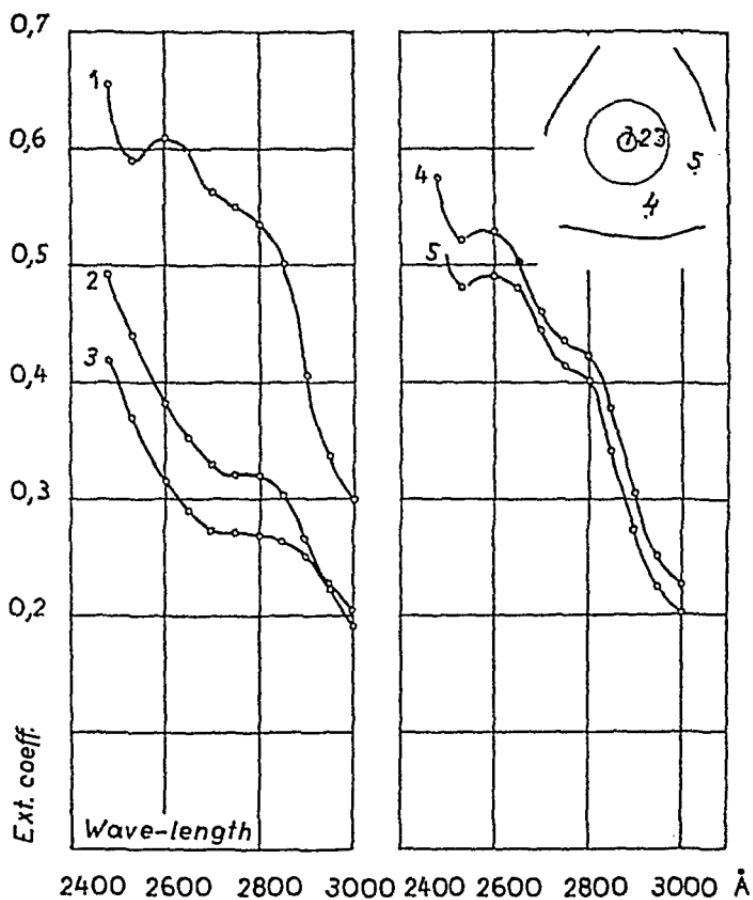


Fig. 19. Absorption spectra from points in the anterior horn cell from a rabbit, illustrated in Fig. 18. The diagram shows the measuring points.

that the nucleolus had a very marked tendency to bind acid dye-groups as compared with other parts of the cell, except the chromocentre (see Chapt. III, p. 45). This shows the markedly basic character of the protein in the nucleolar substance.

*The remaining nuclear substance.* Fig. 19, curves 2 and 3, shows two absorption spectra taken at points in the remaining nuclear substance, curve 2 being from a point near the nucleolus, and curve 3 from a point near the nuclear membrane in the same cell. The measuring points lie in a straight row and the measurements were taken in immediate succession to one another.



Fig. 18. Anterior horn cell, rabbit, frozen-dried. Magnification 1,150  $\times$ . Objective aperture 0.85. Condenser aperture 0.6.



Fig. 20. Spinal ganglion cell, large type, rabbit, frozen-dried. Magnification and optics as in Fig. 18.

HYDÉN: Protein metabolism in the nerve cell.

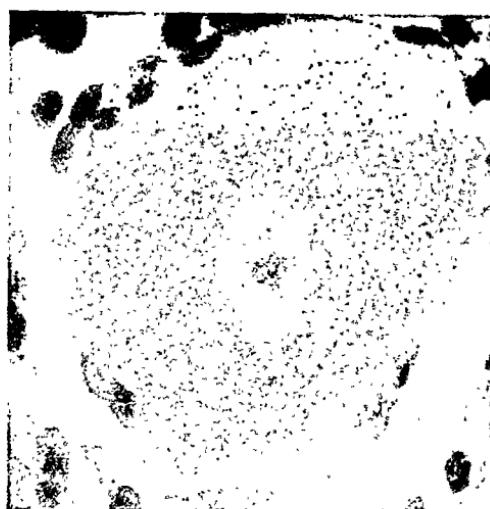


Fig. 22. Spinal ganglion cell, medium-sized type, rabbit, frozen-dried. Magnification 1,150 $\times$ . Objective aperture 0.85. Condenser aperture 0.6.

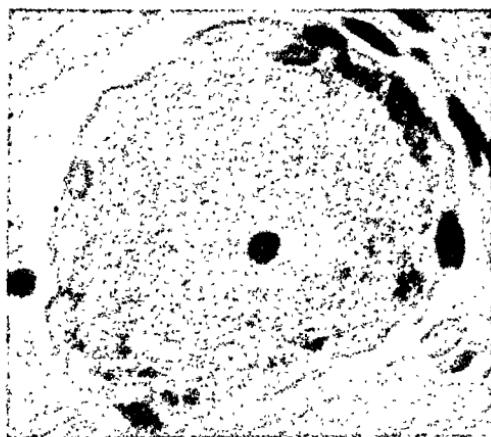


Fig. 24. Spinal ganglion cell, small type, rabbit, frozen-dried. Note that the nuclear substance in this type of cell has a more intense absorptive power at 2600 Å than the large and medium-sized type. Magnification and optics as in Fig. 22.

The ground-substance of the nucleus has another type of absorption than the nucleolus. No distinct nucleic acid band appears, but a low absorption band at 2850 Å dominates. The tyrosin maximum lies near 2900 Å. The tests showed a certain capacity for binding acid dye groups, which however, as compared with the nucleolar substance and the chromocentre, was not marked. An analysis of the absorption spectra 2 and 3 in Fig. 19 shows, however, that the protein concentration is merely about 2—4 per cent, which explains the relatively weak affinity for acid dye groups. The result agrees well with the absorption measurement data, which indicated the occurrence of proteins rich in hexone bases in smaller concentrations. As the height of the specific absorption band is directly proportional to the amount of substance, it will be seen directly from the curves that the content of protein substance is larger nearer the nucleolus than further out towards the nuclear membrane.

*Cytoplasm.* Curves 4 and 5 show some absorption spectra from points at different parts of the intensely absorbing cytoplasm. The curves have very pronounced absorption maxima at 2600 Å, indicating large concentration of nucleotides. At 2800 Å a marked protein band appears. The tyrosin maximum lies at 2870 Å. Staining tests with cyananthrol under standard conditions show that the cytoplasm absorbs acid dye groups with great avidity. This indicates the markedly basic character of the proteins.

The Feulgen reaction showed that the Feulgen-positive elements were accumulated within the nucleus of the cell thus measured. The nucleolus gave a negative result, but at its periphery a number of small rod-shaped particles rich in ribodesose nucleotides appeared. The major part of the nucleolus thus contains ribose nucleotides. Apart from the chromocentre area described in Chapt. III, Part I, p. 42, most of the remaining ribodesose elements in the nuclear substance are situated close to the nuclear membrane in the form of small granules.

The cytoplasm as a whole gave a negative result. This confirms the observation made in previous investigations (see p. 57) that the large concentrations of nucleotides in the cytoplasm of the adult anterior horn cell are of ribose type. Subsequent staining with basic dyes showed that the distribution of the cytoplasmic nucleotides well covered NISSL's picture.

**Micro-incineration** of similar cells from the same material yielded as a result large residues of ash at the site of the nucleolus and

the parts of the cytoplasm intensely absorbing at 2600 Å. These results agreed well with those previously obtained. (See also A. ENGSTRÖM 1943.)

The myorhabdotic anterior horn cell of *Nucleus anterolateralis* in the spinal cord of rats shows the same cytochemical picture throughout. The nucleolus is large and contains high concentrations of ribose nucleotides, as well as very large amounts of protein rich in hexone bases. The nucleus contains proteins rich in hexone bases in small concentrations, and in the majority of the cells the protein concentration is largest close to the nucleolus, diminishing towards the nuclear membrane. The difference, though not large, is quite measurable. The cytoplasm contains large amounts of ribose nucleotides, the disposition of which corresponds to the cytological 'picture of Nissl's bodies. It thus includes remarkably large amounts of protein rich in hexone bases.

This composition and distribution of nucleotides and proteins was shown also by the root cells of rabbits, guinea-pigs and cats.

## 2. Analyses of Spinal Ganglion Cells.

The material consisted of spinal ganglion cells from 16 rabbits. Most of the animals were killed by decapitation, but some of them by embolizing with air through an ear vein. For this purpose a 50 cc Record squirt and a thick needle were employed, whereby it was possible at once to blow in a sufficiently large volume of air in order to kill the animal instantaneously. The spinal ganglia were extracted and immediately fixed. Most of the ganglia were fixed in accordance with Gersh's freezing-drying method; the Carnoy solution was also used.

Altogether some forty ganglia were examined. For measuring in ultraviolet, the author selected such sections as included the major part of the nucleolus, and where the chromocentre area previously analyzed, was so little developed that measuring points in the remaining nuclear substance could be selected in the area from nucleolus to nuclear membrane, which was free from chromocentre substance.

The sections were taken, in the order of enumeration, for ultraviolet microscopy and absorption measurements; the Feulgen reaction; measuring of the capacity for binding acid dye groups; and staining with toluidine blue-erythrosin in accordance with the

usual histological technique. In measuring this material 42 series were arranged in accordance with the methods above described.

In reporting the results, it will be convenient to divide the cells according to their size into three groups. (The maximum and minimum diameter of the *average* cell within each group of the analyzed cells is given in brackets.)

- A. Large cells ( $58 \times 52 \mu$ ).
- B. Medium-sized cells ( $42 \times 38 \mu$ ).
- C. Small cells ( $29 \times 29 \mu$ ).

The results of the investigations of the cells within each of these groups and also of the ganglion cells fixed in different ways were in correspondence. (In regard to the differences in absorption measurements due to the fixing method adopted, see p. 16.) It should, therefore, suffice to adduce, as typical examples, the results of a serial investigation of a spinal ganglion cell from a rabbit, fixed by Gersh's freezing-drying method.

#### A. Large Cells.

**Ultraviolet microscopy.** The cell in Fig. 20 is unstained and was photographed at  $2570 \text{ \AA}$ . The nucleus is spherical and lies centrally in the cell. The large nucleolus absorbs intensely, whilst the nuclear substance in general is marked by a feeble, apparently uniform, absorption. The nuclear membrane is distinguished by a thin, regular, absorbing line. The cytoplasm absorbs more intensely than the nuclear substance, but feebly in comparison with the cytoplasm in the large root cells. The cytoplasm has a zone with a somewhat feebler absorptive capacity in the immediate vicinity of the nucleus and at the periphery of the cell.

**Absorption measurements.** Fig. 20 shows absorption measurements from different points in the cell. The chart indicates the measuring points.

**The nucleolus.** Curve 1 is from a point situated centrally in the nucleolus. At  $2600 \text{ \AA}$  a marked nucleotide band appears. From that point the curve falls towards long wave-lengths, and at  $2800 \text{ \AA}$  a marked protein band is seen. The tyrosin maximum lies at  $2870 \text{ \AA}$ . The results of the measurement of the capacity for binding acid dye groups have been reported in detail in Chapt. III, Part I, p. 45. These investigations showed that the nucleolus had an affinity for acid dyes which far exceeded that

of the other structures in the ganglion cell, except the chromocentre. From this it may be inferred that the nucleolus contains nucleotides and proteins rich in hexone bases in large concentrations.

The remaining nuclear substance. Curve 2 is taken at a point near the nucleolus, and curve 3 at a point near the nuclear

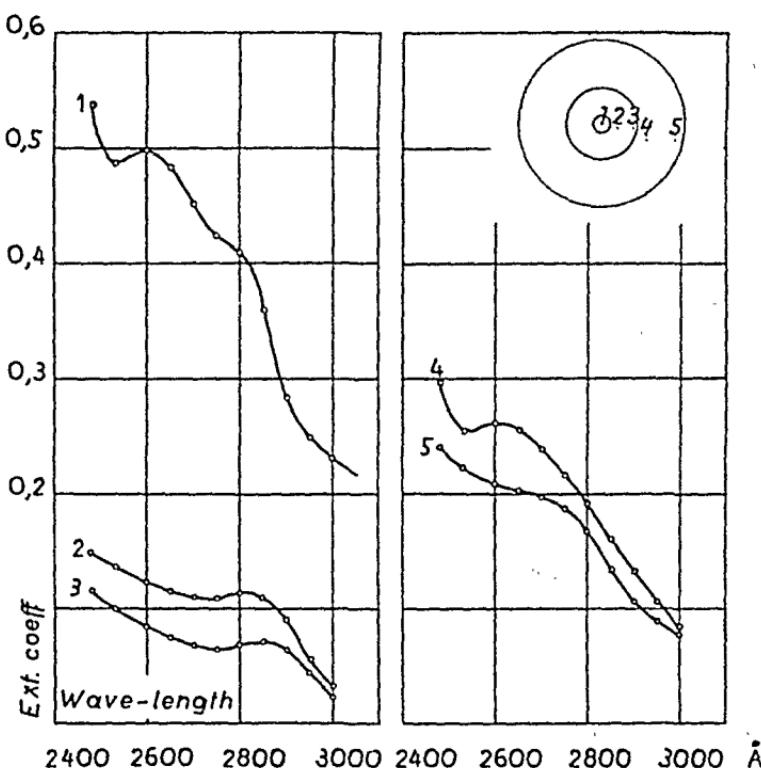


Fig. 21. Absorption spectra from points in the spinal ganglion cell from a rabbit, illustrated in Fig. 20. The diagram shows the measuring points. Curve 5 indicates that the outermost layer of the cytoplasm mainly contains substances with the absorption type of the coagulable proteins.

membrane. Nucleotide bands are lacking in both curves. At 2800 Å weak protein bands appear. The tyrosin maximum in both cases lies near 2900 Å. Investigation of the binding capacity showed that the nucleus substance had a certain tendency to bind acid dye groups. This capacity, however, was slight as compared with that of the nucleolar substance and the chromocentre. This agrees very well with the results of the absorption measurements, and the combined investigation procedure shows that the nuclear substance contains proteins rich in hexone bases in small concentrations.

An analysis of the curves shows that the protein concentration

close to the nucleolus is nearly twice as large as in the vicinity of the nuclear membrane.

*The cytoplasm.* Curve 4, Fig. 21, is taken at a point in the cytoplasm between the nuclear membrane and the periphery of the cell. At 2600 Å a distinct nucleic acid band appears. At 2570 Å there is a protein band, which increases the breadth of the absorption maximum at 2600 Å. The absorption measurement thus shows the occurrence of nucleotides in moderate concentrations as well as proteins with the absorption type of the higher proteins. In the dye-binding tests the cytoplasm showed a very slight tendency to bind acid dye groups, which corresponds very well with the data from absorption measurements.

Curve 5 is taken at a point in the cytoplasm near the periphery of the cell. At 2570 Å a protein band appears, but no nucleotide band. The curve shows the occurrence of proteins of the same type as in the remainder of the cytoplasm.

### B. Medium-sized Cells.

**Ultraviolet microscopy.** Fig. 22 shows a photograph at 2570 Å of a spinal ganglion cell belonging to the group. Apart from the size, it differs from the ultraviolet cytological picture of a cell of the large type merely by the greater absorptive power of the cytoplasm.

**Absorption measurements.** *The nucleolus.* Curve 1 in Fig. 23 is taken at a central point in the nucleolus. At 2600 Å a distinct absorption maximum is seen. Afterwards the curve falls towards long wave-lengths similarly as in the previously described absorption spectra from nerve cell nucleoli, which gives a characteristic appearance to these curves. At 2800 Å a well-marked protein band is observed. The tyrosin maximum is situated at 2850 Å. The dye-binding tests, as in the preceding cases, showed a great affinity for acid dye groups. The results thus indicate, as in the previous analyses, that the nucleolar substance contains nucleotides and proteins rich in hexone bases in large concentrations.

*The remaining nuclear substance.* Curve 2, Fig. 23, is taken at a point between the nucleolus and the nuclear membrane. At 2800 Å a faint protein band is seen, whereas the nucleotide band is missing. The tyrosin maximum is situated at 2900 Å. Dye-binding tests showed a certain affinity for acid dye groups, which, however, was slight as compared with that of the nucleolus.

and chromocentre. This shows that also in this type of spinal ganglion cells the nuclear substance contains proteins rich in hexone bases in small concentrations, whereas nucleotides in any large concentrations could not be shown.

*The cytoplasm.* Curve 3, Fig. 23, is taken at a point in the cytoplasm between the nuclear membrane and the periphery of the

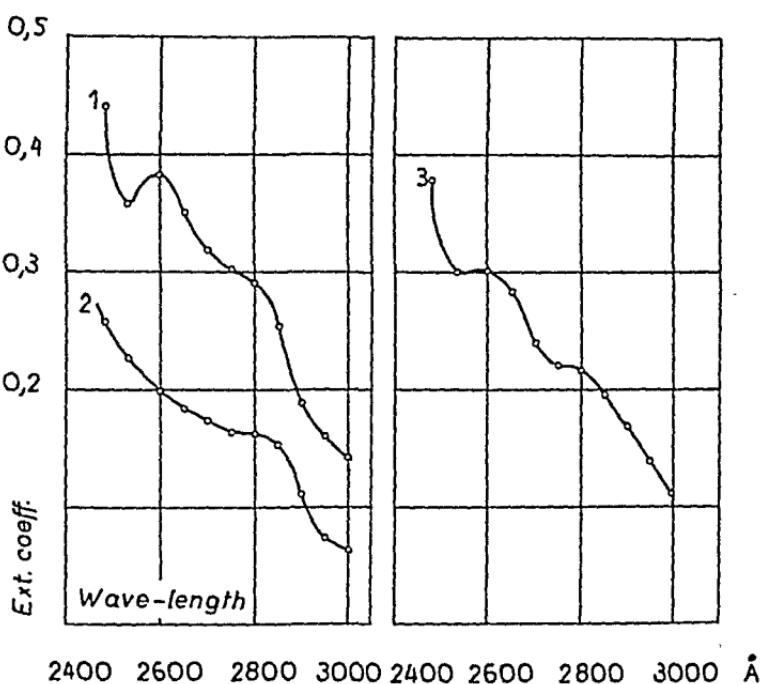


Fig. 23. Absorption spectra from points in a spinal ganglion cell of the medium-sized type, from a rabbit. The nucleolus (curve 1), the nuclear substance between nucleolus and nuclear membrane (curve 2), and from a point in the cytoplasm (curve 3).

cell. At 2600 Å a distinct nucleic acid maximum is seen. At 2800 Å a protein band appears, as a bulge in the profile of the curve. The tyrosin maximum lies at 2850 Å. In cells of this type the affinity for acid dye groups in the cytoplasm was considerably greater than in the cells of the large type. The investigations thus show that the cytoplasm in the medium-sized type of spinal ganglion cells contains nucleotides as well as proteins rich in hexone bases in considerable concentrations.

### C. Small Cells.

**Ultraviolet microscopy.** Fig. 24 shows such a cell photographed at 2750 Å. In the ultraviolet picture it differs from the large and medium-sized type of cells, especially in the fact that the

nucleus is marked by singularly intense absorptive capacity. The cytological picture is otherwise the same.

**Absorption measurements.** The absorption measurement data showed for spinal ganglion cells of this size-group a certain difference in the composition of the nuclei in different cells, in that the nucleus in some of the cells analyzed contained a nucleotide component in concentrations which it was not possible to show in the nuclei of the other cells. In the investigation material consisting of eight thoroughly analyzed series in accordance with the procedure described on p. 60, the nuclei in three cases showed this nucleotide component. Two absorption series for a couple of cells of the small type are given below.

*The nucleolus.* Curve 1 in Fig. 25 is taken at a point situated centrally in the nucleolus. The curve shows the same composition

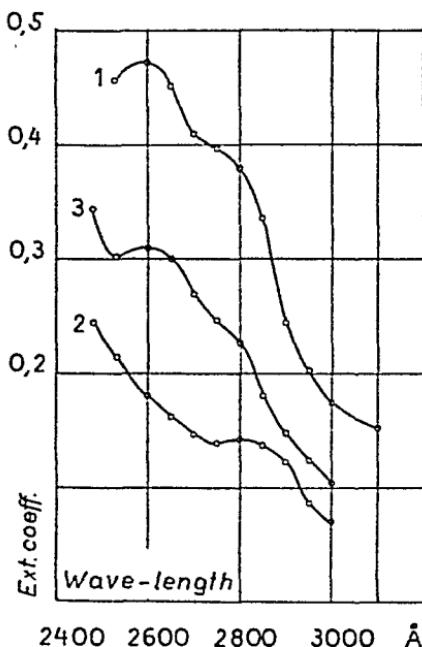


Fig. 25. Absorption spectra from points in a spinal ganglion cell of the small type, from a rabbit. The nucleolus (curve 1), the nuclear substance between nucleolus and nuclear membrane (curve 2) and the cytoplasm (curve 3).

of the nucleolar substance as in the previous types of ganglion cells, and the dye-binding tests also gave similar results: nucleotides and proteins rich in hexone bases in large concentrations.

*The remaining nuclear substance.* Curve 2 in Fig. 25 is taken at a point between the nucleolus and the nuclear membrane. The curves are analogous in shape with those taken at a corresponding point in the large and medium-sized type of spinal

ganglion cells. At 2800 Å a distinct protein band appears, whereas no certain nucleotide band can be shown. The tyrosin maximum lies at 2900 Å. Staining tests showed a capacity for binding acid dye groups which was weak relatively to the nucleolus, but considerable as compared with the ganglion cells of the large and medium-sized type. From this it follows that the nuclear substance contains proteins rich in hexone bases in moderate concentrations, whereas nucleotides in marked concentrations cannot be shown.

*The cytoplasm.* Curve 3 in Fig. 25 is taken at a point in the cytoplasm between the nuclear membrane and the periphery of the cell. At 2600 Å a distinct absorption maximum appears. At 2800 Å a distinct protein band is observed, after which the curve falls rapidly towards longer wave-lengths. The tyrosin maximum is situated at 2850 Å. In the staining tests the cytoplasm showed a capacity for binding acid dye groups which considerably exceeded that of the large and medium-sized cells. The absorption bands moreover lie considerably above those obtained in absorption measurements in the other types of spinal ganglion cells.

The cytoplasm in the cells of the small type thus contains nucleotides and proteins rich in hexone bases in considerable concentrations.

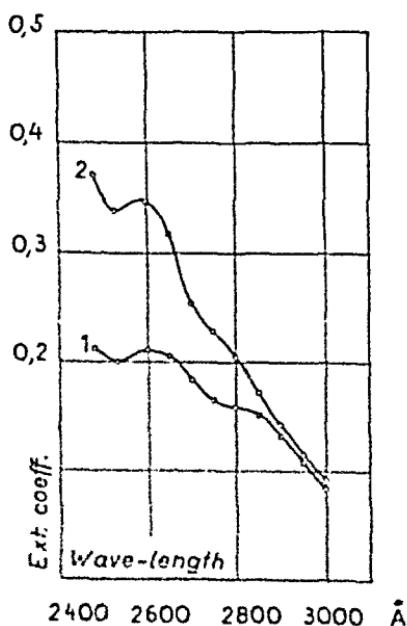


Fig. 26. Absorption spectra from points in a spinal ganglion cell of the small type, from a rabbit. The nuclear substance between nucleolus and nuclear membrane (curve 1) and the cytoplasm (curve 2). Curve 1 shows a nucleotide band, unlike the nuclear substance in the other types of spinal ganglion cells.

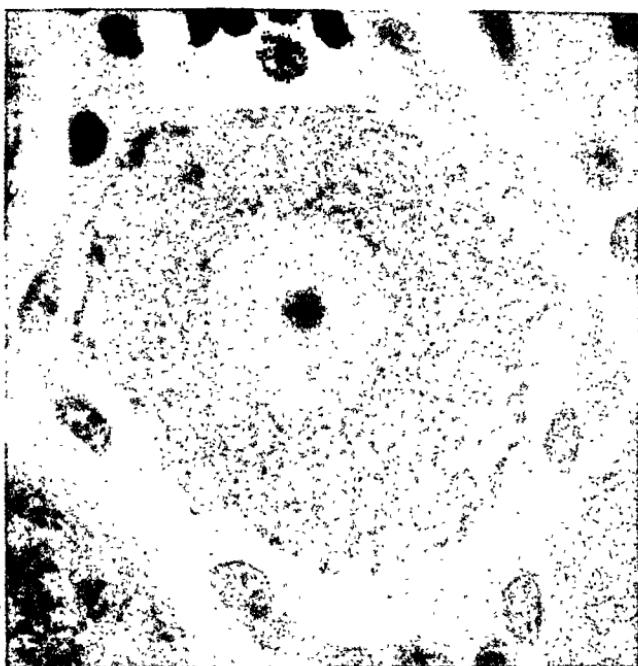


Fig. 27. Spinal ganglion cell, large type, rabbit. Carnoy-fixed. Magnification 1,150 $\times$ . Objective aperture 0.85. Condenser aperture 0.6.

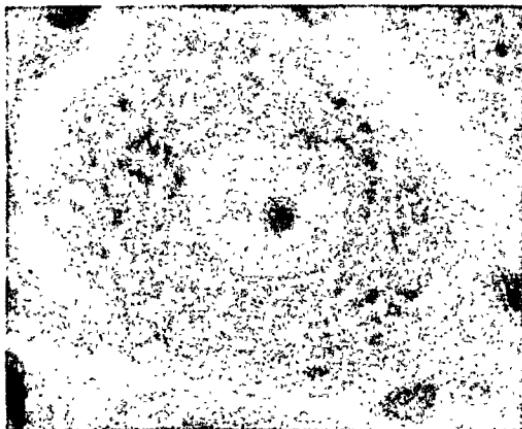


Fig. 28. Spinal ganglion cell, small type, rabbit. Carnoy-fixed. Magnification and optics as in Fig. 27.

HYPDÉN: Protein metabolism in the nerve cell.

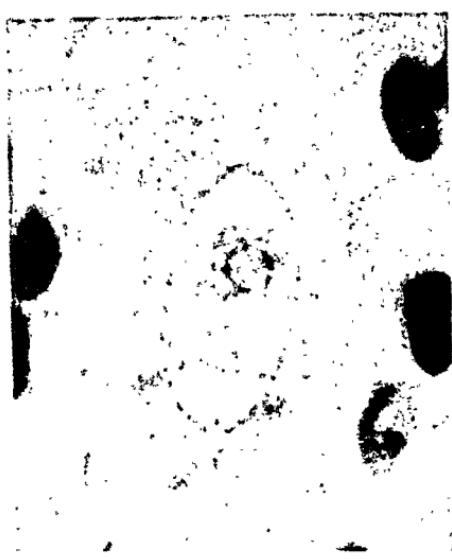


Fig. 29. Spinal ganglion cell, rabbit, on which the Feulgen reaction had been performed. The nucleolus is surrounded by Feulgen-positive particles. Magnification about  $1,200 \times$ .



Fig. 30. Spinal ganglion cell, rabbit, micro-incinerated, photographed in the dark field. Copious residue of ash at the site of the nucleolus, the chromocentre and the parts of the cytoplasm intensely absorbing at  $2,600 \text{ \AA}$ . The peripheral parts of the cytoplasm yield no ash. Magnification  $700 \times$ .

Fig. 26 shows two absorption spectra taken in another cell of the same size-group.

*The nuclear substance apart from the nucleolus.* Curve 1 in Fig. 26 is taken at a point between the nucleolus and the nuclear membrane. At 2600 Å a weak, but distinct, absorption maximum appears. At 2800 Å a distinct protein band is observed. The tyrosin maximum lies at 2850 Å. The staining tests, as in the preceding case, showed a considerable capacity in the nuclear substance for binding acid dye groups. The nucleus in this type of cells thus contains a distinct nucleotide component in addition to proteins rich in hexone bases in considerable concentrations.

*The cytoplasm.* Curve 2, Fig. 26, is taken at a point in the cytoplasm between the nuclear membrane and the periphery of the cell. In analogy with a cell of this group previously analyzed, it shows the occurrence of nucleotides and proteins rich in hexone bases.

#### Carnoy-fixed Spinal Ganglion Cells from Rabbits.

As pointed out in Chapter II, Part I, p. 16, regarding the importance of the fixing for the result of the absorption measurement, an agent which violently precipitates protein, will give a larger loss of light owing to dispersion in the cell material than fixing in accordance with the freezing method. The curves will have a steeper course. No new absorption band will, however, be observed, nor will any absorption maxima be effaced.

Only in this way did the absorption spectra taken on Carnoy-fixed material differ from those reported above on material fixed by the freezing-method. As previously pointed out, those parts which are intensely absorbing at 2600 Å, corresponding to the parts rich in nucleotides, will stand out in particularly sharp relief on the photographic plate. By way of example I show a spinal ganglion cell from a rabbit of the large (Fig. 27) and the small type (Fig. 28).

*Staining with basic dyes.* In the staining of spinal ganglion cells previously photographed in ultraviolet light, a good correspondence between the distribution of the cytoplasmic nucleotides and NISSL's pictures was observed. This confirms earlier results with similar methods (see Chapt. I, Part II, p. 57).

The Feulgen reaction gives one identical result for the different types of ganglion cells here described. The nucleolus as regards

the major part of its mass gives no reaction. An observation made throughout in regard to the ganglion cells is that the Feulgen-positive material is collected in small, rod-shaped elements at the periphery of the nucleolus, which thus contains ribodesose nucleotides. See Fig. 29. On sections which include the major part of the nucleolar mass these rods number as a rule about ten. They correspond merely to an insignificantly small part of the nucleotide mass in the nucleolus, which amounts on an average according to ultraviolet data, to about 2 per cent. In photographing in ultraviolet at 2570 Å, these small particles will be covered by the large numbers of *ribose* nucleotides constituting the main mass of the nucleolus. On some ultraviolet photographs, however, one can observe isolated ribodesose structures at the periphery of the nucleolus, as intensely absorbing rods.

The nuclear substance in general contains a very small amount of Feulgen-positive elements. They lie for the most part in the form of small particles assembled close to the nuclear membrane. On a visual estimate of the number of Feulgen-positive particles in the different types of ganglion cells, they appear to be approximately equal in nuclei of different size. The larger content of nucleotides in some of the nuclei of the small type of cell seems therefore to be mainly due to *ribose* nucleotides. In all the cases the cytoplasm gave no reaction, which signifies that these cytoplasmic nucleotides, as in the other ganglion cells, are of *ribose* type.

**Micro-incineration.** The micro-incineration of the spinal ganglion cell shown in Fig. 30, photographed in the dark field, has yielded a large amount of ash. This ash lies at the place of the nucleolus and the parts absorbing at 2600 Å, which covered Nissl's bodies in the cytological picture. From the above detailed analysis it is evident that the peripheral part of the ganglion cell cytoplasm contained a very small amount of nucleotides. This tallies well with the result of the micro-incinerations, for as shown by the photograph, the peripheral parts of the cell are very poor in ash.

#### Survey of Results.

The spinal ganglion cells from rabbits may be divided into different groups according to size. In the cells of the different groups a characteristic distribution of nucleotides and proteins can also be shown.

The nucleolus contains large concentrations of ribose nucleotides. Furthest out in its periphery one always finds a few small, rod-shaped elements, rich in ribodesose nucleotides. Close to the nucleolus, and often in close contact with it, an irregular structureless nuclear part could be shown, which contained large concentrations of proteins rich in hexone bases as well as smaller amounts of ribose and ribodesose nucleotides. As shown in Chapter III, Part I, p. 42, this nuclear substance is analogous with a chromocentre.

The nuclear substance in general contains proteins rich in hexone bases in small concentrations: they are largest round the nucleolus and diminish towards the nuclear membrane. In some of the ganglion cells of the smaller type, ribose nucleotides could be shown in the nuclear substance, apart from the nucleolus.

The cytoplasm contains ribose nucleotides corresponding to the cytological picture of Nissl's bodies, as well as proteins in moderate concentrations. In the larger ganglion cells these concentrations have the absorption type of the higher coagulable proteins, whereas in the smaller cells, where the concentration of nucleotides is larger, they have a more basic character. A narrow zone furthest out on the periphery contains chiefly proteins.

In order to investigate whether there are any differences in the composition of ganglion cells of the three above-mentioned size-groups, a survey has been made of the total amount of nucleotides and proteins in cell-bodies of these different groups. Results of the measurements of the average cell in each group, fixed by the freezing-drying method, are adduced by way of example. The estimates are based on five absorption spectra from cells in each group.

In computing the cell volume it has been assumed that the ganglion cells are rotation ellipsoids, and the calculations are made as explained on p. 30, and on sections which include the major part of the nucleolus.

The larger type of the spinal ganglion cells examined here, reckoned as rotation ellipsoids, had an average cell volume of  $656,100 \mu^3$ , the nucleus having a volume of  $17,100 \mu^3$ . The ratio of the nuclear volume to that of the cytoplasm was about 1 : 37.

The average volume of the medium-sized cells was about  $260,480 \mu^3$  the nuclear volume  $9,200 \mu^3$ . The ratio of the nuclear volume to that of the cytoplasm was about 1 : 27.

The average volume of the smallest cells was  $90,390 \mu^3$ , the

nuclear volume  $5,570 \mu^3$ : the ratio of the nuclear volume to that of the cytoplasm was about 1:16.

As has also been pointed out in the literature, the nuclear plasma volume index, i. e. the nuclear volume expressed in percentage of the cytoplasmic volume, is higher in the small spinal ganglion cells than in the large ones.

A comparison between the total amount of proteins and nucleotides in the cells of these size-groups shows a considerable difference between the groups.

The table below gives the ratios of the average total amount of nucleotides and proteins in the different types of spinal ganglion cells.

	Small cells	Medium cells	Large cells
Total amount of nucleotides	1	2	3
Total amount of proteins	1	1.5	8

From this it will be seen that the average composition varies in ganglion cells of different size, and that the cells of the large type are distinguished by a very considerable amount of proteins relatively to the two other types of cells, but by a proportionally smaller amount of nucleotides.

### 3. The Nucleolar System in Spinal Ganglion Cells from certain Fishes.

In the previously cited cytochemical investigations of spinal ganglion cells of certain fishes, *Lophius piscatorius*, *Gadus* and *Esox*, an intense activity of the nucleolar cytoplasmic nucleotide system was shown. What renders these cells particularly suited for cytochemical investigations is their size, and an intensive formation of protein in the cytoplasm, to which the nucleolar mechanism is connected. As we are apparently concerned here with a universal cell mechanism (see the preceding chapter), which in the above specified species of fish is unusually well-developed, a brief survey of the results of these investigations will be given here.

Cells of three different types could be shown in the same ganglion (see the accompanying sketch, Fig. 31). The investigations indicated that the different types of cells might be stated, with a great degree of probability, to correspond to different stages in a physiological process. The first stage would then be represented by the cell of type I (*a* in the Figure), which constituted merely



Fig. 32. Spinal ganglion cell from *Lophius piscatorius*, frozen-dried. The nucleus is eccentrically situated and the nuclear membrane is greatly folded within the part facing the centre of the cell. Close to that area there are ribose nucleotides in high concentrations. The remainder of the cytoplasm is poor in such nucleotides.



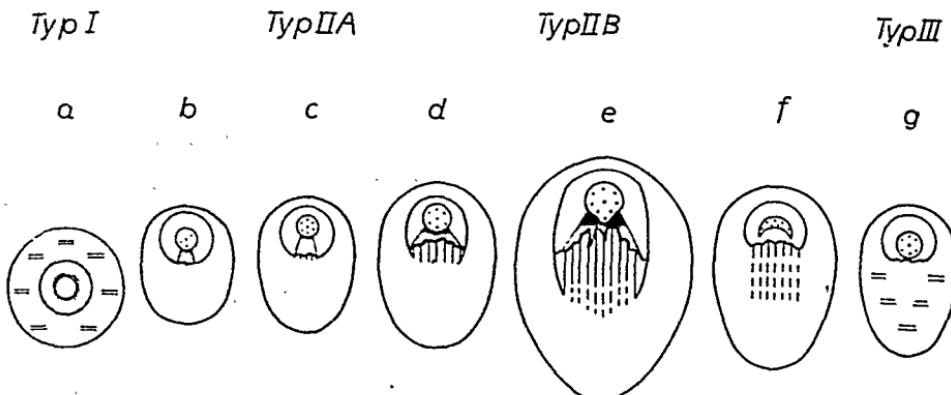


Fig. 31. Diagram showing the different types of cell in a spinal ganglion from *Lophius piscatorius*, corresponding to the different stages in the cytoplasmic protein-formation in the ganglion cell. See the text. The horizontal lines indicate the aggregations of ribose nucleotides in the cytoplasm, the vertical lines the nucleotides of the nuclear membrane. The shading indicates the diminution of concentration in the protein substance from the nucleolus towards the nuclear membrane. (From HYDÉN 1943.)

a few per cent of the total number of cells in the ganglion and on an average had the smallest cell volume. In the nucleus a distinct diminution in the concentration of proteins rich in hexone bases from the nucleolus out towards the nuclear membrane could be observed. In the cytoplasm there were accumulations of ribose nucleotides which in the cytological picture corresponded to Nissl's bodies. In type II A it was possible, through transitional forms (b and c in the Figure), close to a well-defined area of the nuclear membrane, to follow the formation of an accumulation of ribose nucleotides in the cytoplasm, "nuclear-membrane nucleotides". The nuclear membrane within this section was irregularly folded. In the remainder of the cytoplasm the content of both nucleotides and proteins was greatly reduced. On staining, the picture of a chromatolysis was obtained. The nuclear substance in type II A was both absolutely and relatively better developed than in type I, and contained larger concentrations of nucleotides and especially of proteins rich in hexone bases. Between the nucleolus and the nuclear-membrane nucleotides the concentration of proteins rich in hexone bases was several times larger than in other parts of the nucleus. This showed a process in which both the nucleolus and the nuclear membrane were involved. In type II B (Fig. 32 shows an ultraviolet photograph of such a cell) a reduced concentration could be shown in the tongue-shaped, prolonged part of the nucleolus, containing proteins rich in hexone bases and, to a smaller extent, ribose nucleotides,

which indicates a migration of protein substance rich in hexone bases from the nucleolus in the direction of the irregular section of the nuclear membrane. The connection between nuclear-membrane nucleotides and this activity of the nucleolus was indicated by the fact that the former began to be developed at the stages when the characteristic distribution of proteins in the nucleus could first be shown, and reached its greatest development when the nucleolar phenomena were most pronounced.

The correlation between the nuclear membrane nucleotides and the synthesis of the cytoplasmic proteins was apparent from the determinations of the total amounts of proteins in the cells. These showed in fact a marked increase of cytoplasmic proteins during the development from type I through the intermediate stages up to type II. *From this it was concluded that the formation of cytoplasmic proteins was induced by the migration of protein substance rich in hexone bases from the nucleolus out towards the nuclear membrane, where the formation of ribose nucleotides takes place.* In the ganglion cells, type II, of *Lophius piscatorius*, for example, the nucleolar mechanism functions intensively, and in this process a limited part of the nuclear membrane participates. In type I the nucleolar mechanism functions, relatively speaking, in but a slight degree, and the entire nucleus is involved.

Ganglion cells, type II, from other fishes, *GADUS* and *Esox*, were likewise analyzed. They represented merely a small number of the cells. The major part of the ganglion cells resembled those of type I.

The ganglion cells in spinal ganglia from these species of fish are typical examples of cells with an intensively functioning nucleolar mechanism, through which a formation of cytoplasmic proteins is carried on with the aid of basic proteins.

As appeared from the analyses of ganglion cells from rabbits, they have a composition similar to that of the above-described cells of type I.

### Summary, Chapter I.

The adult myorhabdotic nerve cells from the anterolateral group in the anterior horn of different animals show the same cytochemical picture throughout. The same applies to the different types of spinal ganglion cells. All these cells are distinguished by a well-developed nucleolar- cytoplasmic nucleotide apparatus.

## CHAPTER II.

### Cytological Changes in Nerve Cells during Different Functional Conditions.

The results of the investigation of the distribution of nucleotides and proteins in a large number of adult nerve cells from different mammals *at rest* have been reported in the preceding chapter. The results in the various types of cells were throughout in correspondence, and will be referred to in the sequel as "standard organization", as compared with cells in other functional conditions.

Investigations were thus made of motor nerve cells from animals in intense motor activity, on sensory nerve cells from animals exposed to intense irritation of those nerves, as well as on motor and sensory nerve cells after excision of the neurite.

*1. Fatigue tests on motor nerve cells.* MANN (1894) examined the pyramid cells in the motor cortex and the motor nerve cells in the spinal cord of dogs who had been made to perform intense muscular work, carried to complete exhaustion. In the pyramid cells he observed a distinct diminution in the capacity of the cytoplasm to take up basic dyes. In some cells the cytoplasm showed the picture of chromatolysis. Besides this, the cells were remarkably pale. In the motor cells of the spinal cord most of the nuclei stained much more intensely than in the control animal. The nuclear contour was moreover irregular. In the cytoplasm the same picture of chromatolysis as in the pyramid cells was observed. GUERRINI (1899—1902) found the same changes in dogs which had been made to run to complete exhaustion. The anterior horn cells showed a picture of chromatolysis. The nuclei were remarkably large and excentrically situated in the cell. Some nuclei were irregularly shaped. The nucleolus in the cells is de-

scribed by GUERRINI as readily stainable with basic dyes and larger than in the corresponding cells of the control animals.

HOLMES (1903) examined motor cells in the spinal cord of frogs which had been strychnized according to VERWORN. The experimental animals were killed at different times after the spastic cramp had set in. In order to study the effect of a protracted supply of strychnine and lack of oxygen, the blood was replaced by a physiological salt solution and the experimental conditions were kept constant. In the cells the principal changes consisted of a peripheral displacement of the nucleus, and an increase in its size, as well as in that of the nucleolus and the cell-body as a whole. The stainability of the cytoplasm with basic dyes was greatly reduced. HOLMES contended that the nerve cell changes had not been directly caused by a toxic effect of the strychnine, but in all probability were due to the increased motor effort.

Ganglion cells with long axons have on an average a larger volume than those with short axons. According to HEIDENHAIN (1911), there is a distinct correlation between the total amount of basophil substance in the cytoplasm and the plasma volume.

DOLLEY (1913) examined ganglion cells from crayfish which had been made to perform strenuous muscular work. He could observe a distinct increase in the ganglion-cell volume in the exhausted animals.

TERNI (1920) compared the ganglion cells in the last cordal segments and in the cervical segments of the extremities. He found a very considerable difference in size. The cervical cells were 9—23 times larger. Also the shape was different. The cervical cells were more rounded-off and had larger processes than the cordal ones, which were small and narrow. The results indicate the correlation between the size of the innervation area and the ganglion-cell volume.

EINARSSON (1933) examined anterior horn cells of rabbits in which, by a suitable dosage of ether during narcosis, he could produce running movements with "alternating flexion-crossed extension". On dyeing with gallocyanin, the author considered that he could observe that the motor cells which enervated the limbs in rigid extension showed a chromophobe picture, the motor cells of the flexed extremities a chromophile picture. EINARSSON inferred from his experiments that central excitation and inhibition were represented by a nerve cell which was chromophobe and

chromophile, respectively. As for the picture of a parapyknomorphous cell, he held that it corresponded to an "indifferent resting cell".

DÜRKEN (1911) extirpated the rudiments of the extremities in frogs and established that the spinal ganglion cells in question were considerably smaller than under normal conditions. TERNI (1920) made extirpation experiments on SAURIANS and found that on the regeneration of the tail a considerable hypertrophy of the ganglion cells occurred in the segments from which the regenerating parts were innervated. AGDUHR (1920), in working tests on animals, noted a considerable increase in the size of the ganglion cells. These tests argue strongly in favour of a connection between the size and the function of the nerve cells.

*2. Fatigue tests on sensory nerve cells. Light tests.* PERGENS (1897), MANN (1898), and CARLSSON (1902) exposed the retina of dogs and birds to light, and found that the capacity of the ganglion cells for staining with basic dyes was considerably reduced. In the opinion of these authors, the results show that the chromophil substance is consumed during activity and is stored in the cell during rest.

*Electrical irritation* of nerve centres has been employed on a large scale in experimental investigations of nerve cells. The results are throughout fairly uniform. HODGE (1894), VAS (1892), MANN (1894), NISSL (1894, 96—97), PICK (1898), LUXENBURG (1898), HOLMGREN (1899, 1900). On moderate irritation for some minutes an increase in the volume of the cell-body, cell nucleus and nucleolus and a peripheral displacement of the nucleus could be observed. The basophil substance in the cytoplasm diminished. The tendency of the cytoplasm to absorb acid dyes was very considerably reduced, and the basophil substance was seen to be diffusely distributed over the entire cytoplasm. After protracted electric irritation up to ten hours, a decrease in the volume of the cell-body and nucleus could be observed. The nucleus, on being stained, stood out darker than the cell-body, and often had an irregular contour. In the cytoplasm the chromatolysis was still more pronounced.

KORYBUTT-DASKIEWICZ (1899) and HOLMGREN (1900) state that the eosinophil substance in the nucleus increases on electrical irritation. On electrical irritation of sympathetic ganglion cells, according to HODGE (1894) and EVE (1896), changes of the same type, no less pronounced, could be observed. SCARBOROUGH

(1938), on electrical irritation of sympathetic ganglia, was unable to observe any change in the appearance of Nissl's bodies.

LUGARO (1895) gives a careful description of cytological changes and modifications in the volume of ganglion cells of *Gangl. cervicale sup.* in rabbits.

As an irritant LUGARO employed faradic current of a strength "which is scarcely felt on the tongue". On irritation up to half an hour LUGARO found an increase in the largest diameter of the nerve cell with a maximum after five minutes at 6.69 per cent. If the irritation was continued, a reduction could be observed with a maximum after six hours, at 16.53 per cent. The largest diameter of the nucleolus could also be shown to increase after five minutes and amounted to 12.40 per cent. After six hours' irritation a shrinkage of the nucleolus by 1.42 per cent could be observed. Similar, though less marked, changes could be shown in regard to the nucleus.

LUGARO observed an enhancement of the capacity of the cell body for absorbing basic dyes so long as the irritation was accompanied by an increase in the size of the cell. He stated also that the amount of basophil particles had increased, and that this could particularly be observed round the nucleus. After protracted irritation, the cells stained poorly with basic dyes.

LUGARO infers from these experiments that activity is accompanied by an increase in the size of the nerve cell, whilst fatigue is accompanied by a decrease. The correspondence of the cytological changes in the ganglion cells to the functional conditions in the above cited experiments with electrical irritation, was interpreted by PUGNAT (1898, 1901) and VAN GEUCHTEN (1897) briefly as follows:

The morphological indications of the irritation are an enlargement of the volume of cell-body and nucleus, reduction and diffused distribution of Nissl's bodies and a displacement of the nucleus towards the periphery. The exhaustion is morphologically represented by a decrease in the volume of cell-body and nucleus, whilst the nuclear membrane is irregularly outlined and Nissl's bodies become still paler.

EINARSSON (1933) irritated the cells in *Nucleus hypoglossi* faradically for nine hours with a frequency of 20 per second. On subsequent staining with gallocyanin, he observed that in the irritated cells Nissl's bodies were weakly stainable, whereas the nuclei had a greater tendency to take up basic dyes than the nuclei in corresponding non-irritated cells.

3. *Retrograde reaction.* The reaction of the nerve cell after excision of the axon, Nissl's "primary irritation", has been dealt with in a number of works. Extensive studies of this question have been made for example, by NISSL (1892—94), LUGARO (1895), MARINESCO (1897), BALLET and DUTIL (1897) and VAN GEHUCHTEN (1897—1906). Most of these investigations were made on motor cells.

The course of the cell changes can be divided into two phases. During the first phase the chromophile substance in the cytoplasm disappears and, on staining with basic dyes, the nerve cell assumes a diffuse blue tint. Nissl's granules are observed only on the periphery of the cell. According to VAN GEHUCHTEN, this dissolution begins round the nucleus and spreads towards the periphery. According to MARINESCO, the first changes are discerned in the vicinity of the axon hillock. At this stage the nucleus is situated near the periphery of the cell. The nuclear membrane in some cells looks irregular and thickened, and after 24 hours the entire cell assumes a rounded outline; the changes culminate after the lapse of 8—15 days. The recovery phase then gradually sets in. During this phase chromophile substance, first observable round the nucleus, reappears. The Nissl's granules newly formed around the nucleus are readily stainable. After some time the nucleus gradually assumes its previous central position and the cell-body recovers its former outline. The recovery phase seems to terminate after the lapse of 110—120 days.

Thorough investigations of the changes in the number of cells after nerve excision are not available. VAN GEHUCHTEN failed to find any certain difference in the number of ganglion cells in the two hypoglossal nuclei a year after unilateral excision of the peripheral nerve.

That the central reaction depends on the place where the axon is cut had already been shown by the earliest investigations (FOREL 1891, NISSL 1892). The closer to the centre the injury done to the axon, the more serious the cell lesions and the larger the number of cells which degenerate and are phagocytized. WOHLFAHRT and SÄLLSTRÖM (1939) found that the rapidity with which the retrograde cell reaction set in after nerve excision was in proportion to the extent of the excision. If the nerve is totally wrenched out, all the cells degenerate and no cells in the recovery stage can be observed. MARINESCO (1897) attempted to divide the retrograde reaction into two stages according to the position

in the cell where the first chromatolysis was observed. When the dissolution of the chromophile substance could first be observed in the periphery, as after severe intoxications and poisonings (see below), he termed this stage "primary lesion". When the chromatolysis first appeared perinuclearly on the excision of the peripheral nerve, MARINESCO termed this "secondary lesion". This theory was subjected to severe criticism (BALLET 1896, EWING 1898). According to these authors, central dissolution and peripheral displacement of the nucleus could be observed in most cases of "secondary lesion", and no division into primary and secondary lesion could be made. According to VAN GEHUCHTEN (1897), incipient dissolution of chromophile substance should be regarded as a sign of a slowly commencing lesion, and perinuclear dissolution as an indication that the lesion had suddenly set in.

According to VAN GEHUCHTEN, there is a distinct difference between the reaction of the motor and the sensory ganglion cells after excision of the peripheral nerve. He states that in both cases a chromatolytic reaction develops, which in the motor cells passes into a recovery phase, but in the sensory cells is succeeded by degeneration and complete destruction of the cells. According to this author, the explanation was that the motor cells after the excision continued to receive "trophic stimuli", whereas such stimuli were lacking in the sensory cells. LUGARO (1895), in investigations of spinal ganglion cells, found cells in all stages of chromatolysis a fortnight after the excision. He stated that after the lapse of 35 days the number of ganglion cells had greatly diminished. The intact-looking cells predominated and, in his opinion, had been produced by a recovery phase. After excision of the posterior roots he could not observe any retrograde reaction in the ganglion cells. VON GUDDENS (1870) showed that nerve cells in new-born animals were very sensitive to injury of the axon. Excision produced as marked an effect as removal of the whole nerve in adult animals. In new-born animals the retrograde reaction proceeded with great rapidity. VAN GEHUCHTEN noted that a cytological picture similar to that observed in retrograde reaction, with chromatolysis and peripheral localization of the nucleus, could be seen in embryonal cells.

According to HEIDENHAIN (1911), there is a direct connection between the retrograde reaction of the ganglion cell and the peripheral reaction in the nerve. Noting the curious fact that long after a nerve excision a number of ganglion cells may, in

certain cases, be found to have atrophied, whilst the remainder look intact or are in course of recovery, he expressed the view that this depended on whether the growing axons had reached the peripheral nerve-end or not. This author considered that the function of the chromophil substance in the nerve cell was to supplement the activity of the nucleus, whilst the retrograde reaction was due to a disturbance of the nucleus-plasma relation. BETHE (1903) stated that in motor cells an incipient restoration could be observed from the 18th day after the excision of the nerve, even if the nerve-endings did not heal.

According to SPIELMEYER (1922), the recovery-phase pictures in the motor cells of the spinal cord disappeared during the course of the first year after the amputation of an extremity. This author insisted that the changes in the nerve cell had no connection with the regeneration in the peripheral parts. If a central axon was ruptured, the ganglion cell might be restored, although there would be no regeneration of the interrupted course.

SPATZ (1920) considered that the process in the nerve cell during the retrograde reaction consisted mainly in the absorption of fluids in the cell and its consequent swelling. According to that author, the process in the axis-cylinder is analogous with the swelling of the nerve cell. MARINESCO (1930) likewise considers that the chromatolysis picture is due to the absorption of fluids in the cell.

In regard to the exact nature of the retrograde reaction there is a divergence of opinion. According to NISSL and MARINESCO, it is due to atrophic changes. VAN GEHUCHTEN contends that it results from a simple cell disturbance which has affected the chromophile substance, a process to which the nerve cell reacts. SPATZ and SPIELMEYER share this view.

*4. Poisoning tests and investigations.* Cytological changes similar to those observed in retrograde reaction have been noticed after poisonings and in other pathological conditions (EWING 1898, GOLDSCHIEDER and FLATAU 1898, VAN GEHUCHTEN 1906, SJÖWALL 1903, SPIELMEYER 1922, BIELSCHOWSKY 1935). The cytological picture in these various conditions corresponds well to Nissl's designation: acute and grave nerve-cell lesion. In both these forms the picture of a more or less marked chromatolysis can be observed. The "grave nerve-cell lesion" is marked particularly by a distinct change in the stainability of the nucleus, which is rapidly enhanced so that the nucleolus can scarcely be distin-

guished. Round the nucleus in the cells thus changed, caps of basophil substance can be observed. SPATZ (1923), however, questions whether these formations are analogous with Nissl's bodies, as suchsin light green stains them red and not, as the Nissl's bodies, green.

One of the most thorough cytological investigations in this field is SJÖWALL's (1903) investigation of the nerve cells in the spinal cord from a case of tetanus in man, which, after three days' increasing convulsions, went *ad mortem*. All the motor anterior horn cells showed distinct morbid changes. The cells appeared rounded and inflated. The nuclei in most cases were near the periphery of the cell. The nucleolus was considerably enlarged. In cells with eccentrically situated nuclei a flattening of the contour was observed towards the centre of the cell. Within the flattened area the nuclear membrane was basophil, in contradistinction from the other parts, where it was acidophil. In the cytoplasm a severe chromatolysis prevailed. In some of the cells these processes seem to have gone further, in that the cells showed a concavity in the outline of the nucleus. The nuclear membrane within that section was irregularly spinose. In the adjacent cytoplasmic area there was an accumulation of basophil substance, which, with its intense coloration, glaringly contrasted with the surrounding cytoplasmic mass. In SJÖWALL's opinion, these pictures show how the nucleus, by morphologically observable changes, participates in the cellular activity. The accumulations of basophil substance in the vicinity of the peripherally situated nuclei are considered by him to be Nissl's bodies newly formed by the nucleus. SJÖWALL's pictures greatly resemble the cells changes in *Lophius piscatorius* described by HOLMGREN (1899), and he endorses HOLMGREN's view that similar pictures are indicative of the new formation of Nissl's bodies in the vicinity of the nucleus.

The view that chromatolysis in nerve cells is to be regarded as a transitory cell reaction, and not as any form of degeneration, is very strongly supported by GOLDSCHEIDER's and FLATAU's poisoning tests with lead, arsenic, malonitrile, etc. By a suitable dosage of malonitrile it was found possible to produce marked chromatolysis, which after a time could be shown to disappear. The animal thus poisoned recovered from the acute symptoms after the lapse of a few minutes. Even during this acute stage the animal was able to perform all kinetic functions.

In tests with tetanus toxin, GOLDSCHIEDER and FLATAU found characteristic changes in the motor cells of the spinal cord. The chronological order was the following. First appeared a distension of the nucleus, which, on staining with basic dyes, looked paler than in the control animals. While this distension of the nucleus was proceeding, a swelling of Nissl's granules was observed, which, after it had attained a certain degree, was succeeded by a typical chromatolysis.

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## CHAPTER III.

### Nucleotide and Protein Metabolism in Nerve Cells in Motorially Exhausted Animals.

As a first step in the experimental investigations, the effects of muscular work on the nerve cell were examined. The irritations which affect the ganglion cell correspond to those received by the cell under physiological conditions, which affords an opportunity for correlating the changes in the cell with its function.

The laboratory animals were eight young guinea-pigs, four of which were used as control animals. They were made to run in a working-machine until complete exhaustion set in, which as a rule was the case after about two hours. They had not previously been tested in this manner.

These animals were killed immediately after the end of the test by decapitation. The lower part of the cervical and lumbar intumescence as well as the upper part of the sacral cord were taken for examination. Fixation in Carnoy's solution. The root cells lying in the *Nucleus anterolateralis* of the myorabdotic group, which innervate the upper extremity muscles and in these segments stand out well-defined, were used for the investigations. For purpose of comparison, corresponding cells in the control animals were employed. The cells were analyzed in accordance with the procedure described in Chapter I, Part II, p. 60. Only those sections which included the major part of the nucleolus were selected for analysis. Altogether 16 measurement series were arranged, namely 8 on cells from resting animals and 8 on cells from motorially exhausted animals.

The results of the tests on the different animals in each group were in correspondence. A serial investigation of (1) an animal at rest and (2) a motorially exhausted animal reported typical examples.

### 1. Animals at Rest.

**Ultraviolet microscopy.** A myorabdotic cell of this nature is shown in Fig. 33. The nucleus has a rather considerable absorptive capacity, the nucleolus absorbs intensely, and the cytoplasm as a whole has an absorptive power comparable with that of the nucleolus.

**Absorption measurements.** Curve 1 in Fig. 34 is taken at a point in the nuclear substance between the nucleolus and the nuclear

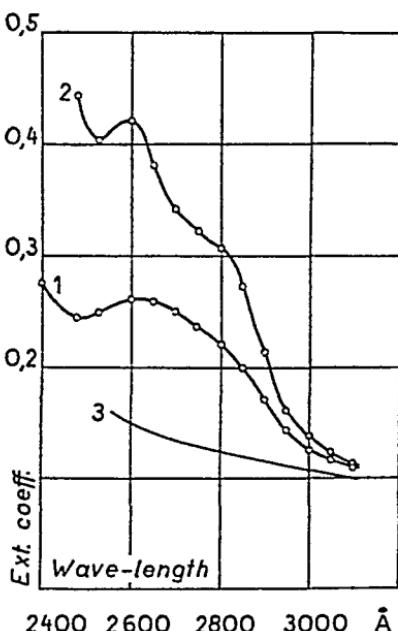


Fig. 34. Absorption spectra from points in the nucleus (curve 1) and cytoplasm (curve 2) in an anterior horn cell from a *relatively inactive guinea-pig*. Curve 1 shows a faint nucleotide band and a marked protein band. Curve 2 shows large concentrations of nucleotides as well as proteins with a tyrosin maximum shifted towards long wave-lengths. Curve 3 indicates the loss of light by scattering.

membrane. The curve is devoid of a distinct nucleic acid band, but has a broad absorption maximum at 2750 Å. The tyrosin maximum lies at 2850 Å. The absorption spectrum thus shows the occurrence of proteins in moderate concentrations, whereas nucleotides do not occur in any appreciable numbers. In regard to the dye-binding tests see below.

Curve 2 is taken at a point in the intensely absorbing cytoplasm. At 2600 Å a marked absorption band appears, which indicates the occurrence of nucleotides in high concentrations. The

curve then falls towards lower wave-lengths, forming a bulge at 2800 Å, after which it drops steeply towards 3000 Å. The tyrosin maximum lies near 2900 Å. The dye-binding tests are reported below.

**Feulgen reaction.** The Feulgen-positive particles are confined to the nucleus, where they are accumulated in the vicinity of the nucleolus and close to the nuclear membrane. In well-fixed sections a collection of Feulgen-positive particles can be observed at the place of the chromocentre.

**Micro-inclination.** The residue of ash in these cells is very copious, and the distribution of the ash corresponds well to the parts rich in nucleotides highly absorbing at 2600 Å.

## 2. Exhausted Animals.

**Ultraviolet microscopy.** All the ganglion cells in the anterior horns are marked by a smaller absorptive capacity at 2600 Å than those of the control animals. An example of a cell from *Nucleus anterolateralis* is shown in Fig. 35. In the nucleus the large, intensely absorbing nucleolus stands out in relief against the remainder of the nuclear substance, which shows but a small absorptive power. The cytoplasm, as compared with that of the control animal, has merely small amounts of absorbing substance. The cell as a whole has more rounded contours, but in serial sections of those cells the author was unable to observe any distinct difference in size between the cells of the exhausted animals and those of the control animals.

**Absorption measurements.** Some absorption spectra are shown in Fig. 36. Curve 1 is from a point in the nucleus between the nucleolus and the nuclear membrane. Near 2900 Å a weak absorption maximum is observed, but above it the curve shows merely a non-specific absorption, which rises slowly towards short wave-lengths. No nucleotide band can be found. The curve analyses of the absorption spectra showed for the nucleus on an average a very low protein content. Curves 2 and 3 are taken at points in the cytoplasm. In curve 2 a protein band is seen at 2750 Å, whereas in curve 3 merely a very weak protein absorption is observed at that wave-length. None of the curves show any absorption of nucleic acid. The curve analyses in the 8 measurement series showed for the cytoplasm a low average content of proteins. The staining tests are reported below.



Fig. 33. Anterior horn cell of *Nucleus myorhabdoticus* from guinea-pig in relative inactivity, Carnoy-fixed. In the cytoplasm large amounts of ribose nucleotides. Magnification 1,150  $\times$ . Objective aperture 0.85. Condenser aperture 0.6.



Fig. 35. Anterior horn cell of *Nucleus myorhabdoticus* from exhausted guinea-pig. Carnoy-fixed. Merely small amounts of nucleotides in the cytoplasm. Magnification and optics as in Fig. 33.



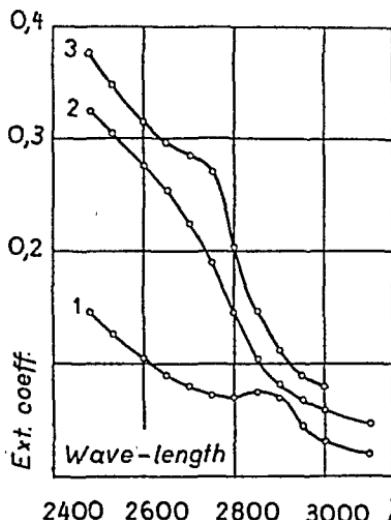


Fig. 36. Absorption spectra from points in the nucleus (curve 1) and cytoplasm (curve 2) in an anterior horn cell from an *exhausted guinea-pig*. Curve 3 is from a point in the cytoplasm of a similar cell. The nuclear substance contains merely small concentrations of protein rich in hexone bases, and the cytoplasm is extremely poor in nucleotides as well as in proteins.

**Feulgen reaction.** In cells from the exhausted animals the Feulgen reaction shows a picture which in no ways differs from that of the control animals.

**Micro-incineration.** The ash content in these cells is strikingly small compared with that of the control animals. At the place of the nucleolus a large residue of ash is observed. Merely small amounts of ash are found in the cytoplasm.

**Capacity for binding acid dye groups.** The preparations were stained with cyananthrol, and the degree of staining was controlled by determining the average amount of bound dye in the erythrocytes. The nucleolus in a number of anterior horn cells of control animals showed on measurement an average extinction coefficient of 0.24. The nucleolus in ganglion cells from exhausted animals gave an average value of 0.33. This shows that the amount of basic groups in the protein of the nucleolar substance is larger in the last-mentioned animals than in the nucleoli of the control animals, which indicates an irritation of the nucleolar apparatus. The nucleus in cells from the control animals gave the extinction coefficient 0.09, and the nucleus in the cells of the exhausted animals showed an average value of 0.15. This tallies well with the absorption measurement data, and the combined procedures thus show that the nuclear substance in the control animals contains proteins rich in hexone bases in large

concentrations, whilst the nuclei in the cells of the exhausted animals contain only small concentrations of those proteins. The cytoplasm in cells from the control animals and the exhausted animals gave on an average the extinction coefficients 0.15 and 0.6, respectively. The results tally completely with those obtained in the absorption measurements. The nerve cell cytoplasm in the motor anterior horn cells from animals at rest contain large amounts of proteins rich in hexone bases relatively to the corresponding cells of exhausted animals.

### 3. Survey of Results.

The myorabdotic ganglion cells from the *Nucleus anterolateralis* of a guinea-pig *at rest* are marked throughout by a high content of nucleotides and proteins. In addition to a nucleolus rich in ribose nucleotides, the nucleus contains proteins rich in hexone bases in considerable concentrations. Diffusely distributed in the cytoplasm, there are very large amounts of ribose nucleotides as well as proteins rich in hexone bases in large concentrations.

The corresponding cells in *exhausted animals* show striking differences in their composition. In the nucleus there is a remarkably large nucleolus rich in ribose nucleotides, which on an average contains larger amounts of basic dye groups than that of the control animals, thus indicating an irritation of the nucleolar apparatus. The remainder of the nucleus contains proteins rich in hexone bases in small concentrations. In contradistinction from the resting animals, the cytoplasm contains merely small accumulations of ribose nucleotides and scanty amounts of proteins, which show but a small tendency to bind acid dye groups.

The author was unable to show any certain disparity in size between the cells of resting and exhausted animals. As appeared from the determinations, the differences in the content of nucleotides and proteins are very considerable, being 3—5 times the respective amounts. If this were due to disparities in cell volume, it would, of course, immediately be shown by the cell pictures, as in that case the differences in volume would be very marked. It is also fairly evident that the large differences cannot be due to variations in the thickness of the sections, as they were observed throughout in the cells of different sections in the 16 series analyzed.

It appears from the above reported results that during intense muscular work with enhanced functioning of the respective myorabdotic cells disintegration of the nucleotides and proteins takes place. This shows that the function of the nerve cell is accompanied by extensive protein and nucleotide metabolism processes. The nucleus in the cells of the exhausted animals contains merely small amounts of proteins, which comprise basic groups in considerable amount. The changes in the cytoplasm go in the same direction. The nucleolus in these cells is remarkably large and rich in ribose nucleotide, and contains proteins with a larger amount of basic groups than that of the control animals. According to previous investigations of ganglion cells (see Part I, Chapter III, p. 39), a large nucleolar apparatus and a preponderance in the nucleus of proteins rich in hexone bases are considered as an indication of an intensively functioning protein-forming system in the cell. The experiments thus show that the changes in chemical composition which entail intense activity of the protein-forming system of the cell can also be induced by the physical irritation involved in increased muscular work. Cytologically, we see here the picture of a slight chromatolysis in the ganglion cells from exhausted animals. The results of the investigations thus show that behind this cytological picture there lies a very marked decrease in the content of nucleotides and protein substance in the ganglion cell. Similar results were obtained in investigations of the ganglion cells of fish (HYDÉN 1943).

### Summary, Chapter III:

It appears from the above reported results that during intense motor functioning the proteins in the nerve cell disappear. The nucleolar apparatus in the cells thus modified shows signs of an intense irritation, which indicates that, though the cell mechanism is adjusted for recovery processes, they appear to be unable, owing to the increase motor functioning, to make good the protein loss.

## CHAPTER IV.

### Nucleotide and Protein Metabolism in Nerve Cells after Electrical Irritation.

The material consisted of spinal ganglion cells from cats (3 animals) and from rabbits (27). Altogether 33 ganglia were examined, besides the control ganglia. The ganglia were exposed during the operation and were irritated with faradic current either pre- or post-ganglionarily. The ganglion on the opposite side in the same segment had previously been removed for purposes of control. The material was fixed firstly in accordance with Gersh's freezing-drying method, and secondly in Carnoy's solution. The current strength was maintained at 1—2 MA. The time was varied from 1 minute up to 10 minutes. The time proved to be of importance for the character of the changes. As a precise grading of the irritation for obvious reasons was impracticable, the results were divided into two groups only, viz. changes after moderate irritation (up to 5 minutes) and after protracted irritation (up to 10 minutes).

The cells were analyzed in accordance with the procedure described in Chapter I, Part II, p. 60. Altogether 42 analysis series were arranged. Only those sections which included the major part of the nucleolus were selected for examination.

The results were in correspondence throughout. A series of analyses from spinal ganglion cells of the large, medium-sized and small groups, which had been irritated faradically for 5 minutes, as well as a serial investigation from a medium-sized cell of a ganglion on which the electrical irritation had been extended to 10 minutes, are reported below as typical examples.



Fig. 37. A couple of ganglion cells, rabbit, electrically irritated for 5 minutes, Carnoy-fixed, containing large amounts of ribose nucleotides. Magnification 1,150 $\times$ . Objective aperture 0.85. Condenser aperture 0.6.



Fig. 38. Another example of a cell electrically irritated for 5 minutes. Carnoy-fixed.

HYDÉN: Protein metabolism in the nerve cell.



Fig. 39. Spinal ganglion cell, rabbit, electrically irritated for 5 minutes. Carnoy-fixed. Close to the excentrically situated nucleus there are large amounts of nucleic-membrane nucleotides. Magnification and optics as in Fig. 37.

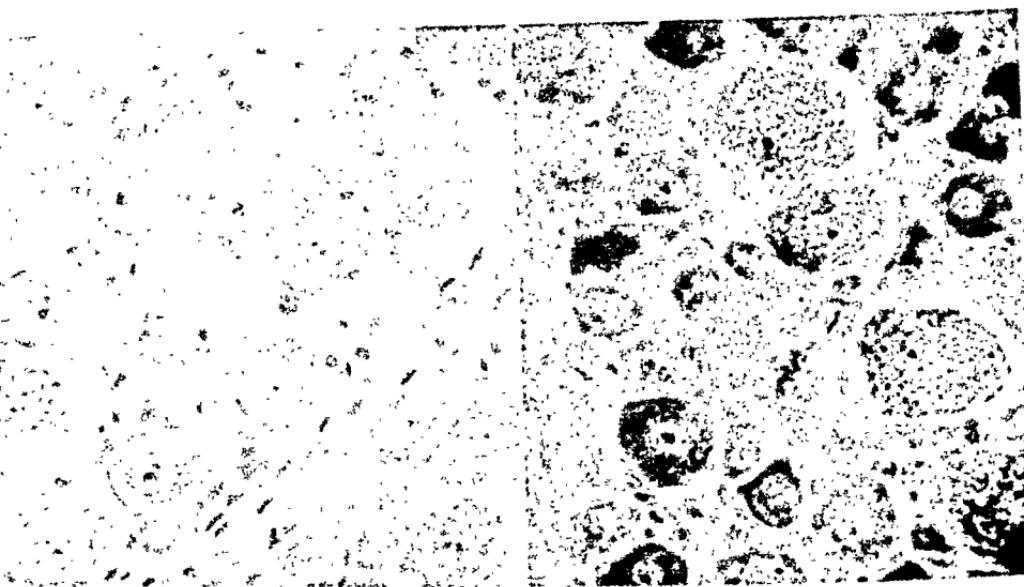


Fig. 45. Spinal ganglion cells, rabbit, non-irritated, dyed with toluidine blue in the presence of surface-tension reducing substances. Magnification about 350 X.

Fig. 46. Electrically irritated spinal ganglion cells stained in the same way as those in Fig. 45. Note the marked difference in the absorption of the stain. The same exposure of both plates and uniform copying on Normal paper. Magnification about 350 X.

## 1. Electrical Irritation up to 5 Minutes.

**Ultraviolet microscopy.** In the vast majority of the cells the ultraviolet cytological picture at 2570 Å shows very considerable deviations from that of the non-irritated cells. Examples of irritated spinal ganglion cells from rabbits, fixed in Carnoy's solution and photographed at 2570 Å, are given in Fig. 37. Most of the cells in such a ganglion show this picture. The cells continue to absorb very intensely. The nucleolus is indistinctly observable in the dark nuclear substance on the photograph. The sharply defined accumulations of nucleotides in the cytoplasm of non-irritated cells, corresponding to the cytological picture of Nissl's bodies, are not seen here. The entire mass of cytoplasm is taken up by large accumulations of absorbing substance, which appear to form a connected whole. Another typical example of a medium-sized, electrically irritated spinal ganglion cell is given in Fig. 38. In the nucleus intensely absorbing, irregular areas are observed in the vicinity of the nucleolus.

Fig. 39 shows a typical cytological picture, particularly characteristic of irritated ganglia. This picture is sometimes observed in the cells of non-irritated ganglia, but is very unusual there. The nucleus is eccentrically situated and flattened towards the centre of the cell. Close to that section of the nucleus the cytoplasm absorbs with great intensity at 2570 Å.

In measuring the average cross-sectional area of the moderately irritated and the non-irritated spinal ganglion cells in central sections comprising the nucleolus, no difference in size could be observed.

**Absorption measurements.** Figs. 40, 41 and 42 show examples of absorption of absorption spectra taken at points in the nucleus between the nucleolus and the nuclear membrane (curve 1) and in the cytoplasm (curve 2) of the large, medium-sized and small type, respectively, of spinal ganglion cells from rabbits. The absorption spectra in Fig. 40 are from a cell fixed by the freezing-drying method, whilst those in the other figures are from Carnoy-fixed cells. All the curves for the nuclear substance show an absorption maximum at 2600 Å. At 2850 Å a marked protein band appears. In all three cases the tyrosin maximum lies at 2900 Å, which indicates that the protein is of basic character. This shifting of the tyrosin maximum is particularly marked in

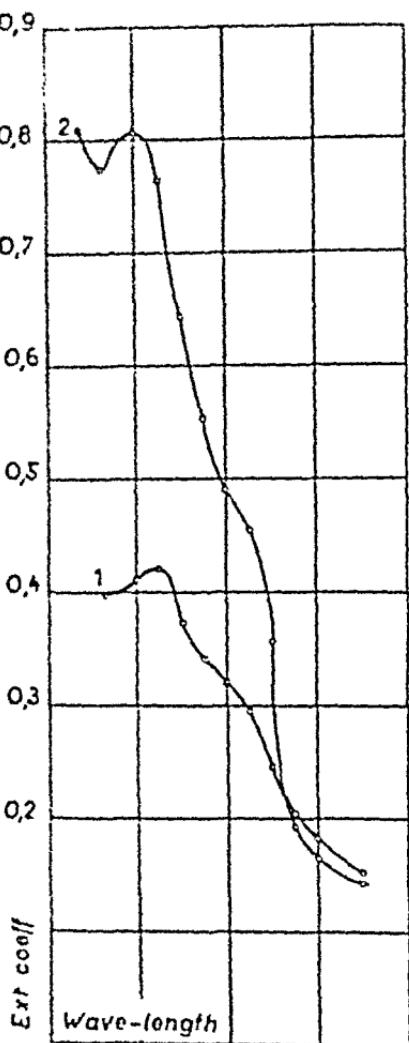


Fig. 40. Absorption spectra from points in the nuclear substance (curve 1) and the cytoplasm (curve 2) in a spinal ganglion cell of the large type, from a rabbit, electrically irritated for 5 minutes.

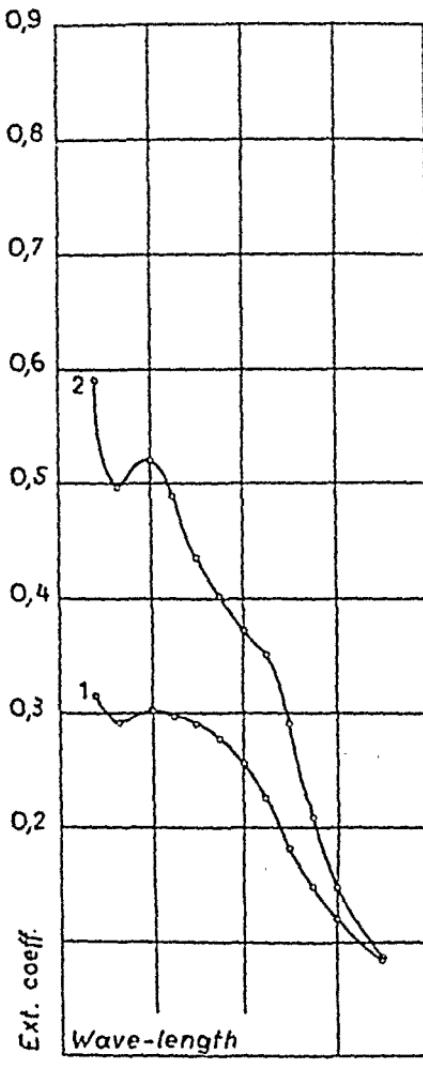


Fig. 41. Absorption spectra from points in the nucleus (1) and cytoplasm (curve 2) in an electrically irritated spinal ganglion cell of the medium-sized type, from a rabbit.

the nuclear substance of the medium-sized and small cells. All the absorption spectra from the cytoplasm show very high nucleic acid bands as well as protein bands with tyrosin absorption maxima shifted towards long wave-lengths, indicating that the protein is of basic character. Particulars regarding the capacity for binding acid dyes are given below.

On comparing the results of absorption measurements of cells from irritated and non-irritated spinal ganglia (altogether 72 se-

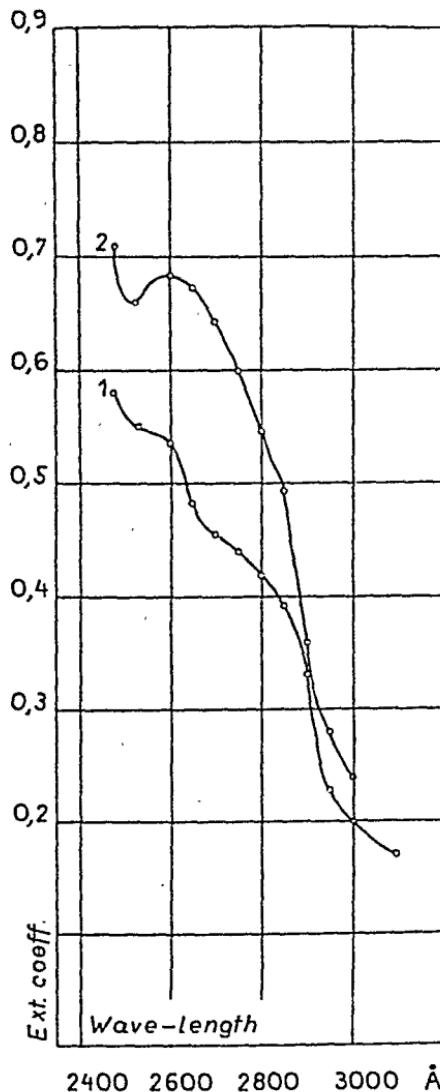


Fig. 42. Absorption spectra from points in the nucleus (curve 1) and cytoplasm (curve 2) in an electrically irritated spinal ganglion cell of the small type, from a rabbit.

ries), very marked differences were observed. The absorption maxima on an average are two to three times higher for the irritated cells than for the non-irritated, and the proportion of nucleic acids to proteins is entirely different.

A comparison of the quotients  $\epsilon_{260}/\epsilon_{280}$  shows that the nucleotides form a considerably larger part of the cell substance in the cytoplasm in the irritated cells than in the non-irritated. The quotient for the non-irritated cells averages 1.3, whilst the corresponding value for the electrically irritated cells is 1.6. Reckoned for "standard protein", this signifies that, whereas

the ratio of the nucleotides to the proteins is 1 : 20 in the non-irritated cells, it is 1 : 5 in those electrically irritated.

The measurements of the cells thus compared were made on sections 5  $\mu$  in thickness, sliced with a Leitz sliding microtome and treated in exactly the same way. Possible variations in the thickness of the slices were not checked; but, as the marked differences in the content of absorbing substance were observed throughout in all the 42 series and in different preparations, they cannot be attributed to incidental inequalities in the sections.

The measurements made by LINDERSTRÖM-LANG and collab. (1935) of inequalities in the thickness of the sections in paraffin slicing with a Minot microtome showed that they did not exceed 0.3  $\mu$  in sections 10  $\mu$  thick.

The results of an analysis of absorption spectra in the cytoplasm of a non-irritated spinal ganglion cell (absorption curve 4 Fig. 21 p. 62) and a corresponding electrically irritated cell (absorption curve 2 Fig. 40 p. 90) are adduced by way of example. The cytoplasm in the irritated cell shows an average nucleotide concentration of 6 per cent, whereas that of the non-irritated cell barely amounts to 1 per cent. The protein concentration is nearly 4 times as large in the cytoplasm of the irritated cell as in that of the non-irritated.

**Feulgen reaction.** The Feulgen-positive elements in the electrically irritated cells completely correspond with the same substance in the non-irritated. They are situated in the nucleus and are arranged in the main perinuclearly as well as close to the nuclear membrane. In a number of cells an accumulation of Feulgen-positive substance at a place corresponding to that of the heterochromatic protein substance was distinctly observed. On a visual estimate of the amount of Feulgen-positive particles, no difference between the irritated and non-irritated cells could be detected. This indicates that the nucleotides which were found in large concentrations in the nuclei of the electrically irritated cells were of ribose type.

**Micro-incineration.** The residues of ash in the electrically irritated and non-irritated spinal ganglion cells differed very considerably. Fig. 43 shows an outline view, in the dark field, of a micro-incinerated section from a non-irritated ganglion, whilst such a section from an irritated ganglion is shown in Fig. 44. The difference of the ash content in the two groups of cells is very marked. In the nuclei and cytoplasms of the irritated

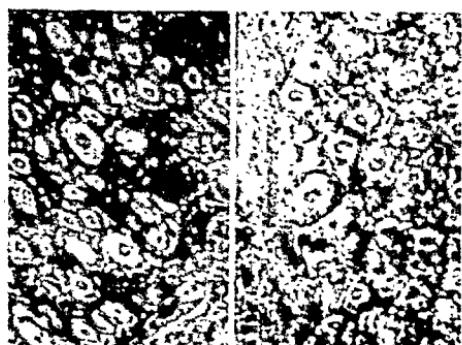


Fig. 43. Micro-incinerated spinal ganglion cells, rabbit, non-irritated, in the dark field. Magnification 220  $\times$ .

Fig. 44. Micro-incinerated spinal ganglion cells, rabbit, electrically irritated for 5 minutes. Magnification 220  $\times$ .

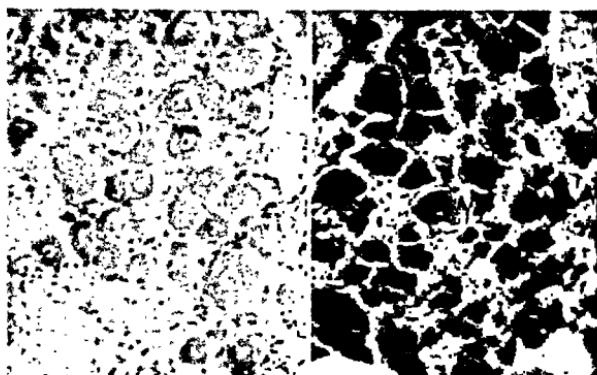


Fig. 48. View of spinal ganglion cells, rabbit, at 2570 Å non-irritated. Carnoyfixed. Magnification 200  $\times$ . Objective aperture 0.20. Condenser aperture 0.20.

Fig. 49. Spinal ganglion cells, rabbit, electrically irritated for 5 minutes, photographed in the same plate as the cells in Fig. 48 and uniformly copied. The same magnification and optics as in Fig. 48.



Fig. 47. Spinal ganglion cell, rabbit, electrically irritated for 10 minutes, Carnoy-fixed. Such large amounts of ribose nucleotides in the nucleus that the nucleolus is completely covered. Cytoplasm poor in nucleotides. Magnification 1,150 $\times$ . Objective aperture 0.85. Condenser aperture 0.6.

cells large amounts of ash are observed, which corresponds well with the localization of the parts intensely absorbing at 2600 Å. The nuclei of the non-irritated cells, on the other hand, yield scarcely any ash, and the ash residue from the cytoplasms is likewise scanty.

**Capacity for binding acid dyes.** In comparing the irritated and the non-irritated cells, the degree of staining in the different sections was checked by computing the amount of dye bound in the erythrocytes. In the non-irritated cells the extinction coefficient thus obtained averaged 0.28 for the nucleolus, 0.09 for the nucleus and 0.13 for the cytoplasm. In the electrically irritated cells the corresponding values averaged 0.40 for the nucleolus, 0.34 for the nucleus and 0.20 for the cytoplasm.

The amount of basic groups in the protein substance of the irritated cells is considerably larger than that of the non-irritated. It is significant that this increase in the content of basic proteins is particularly noticeable in the nucleus and nucleolus. As regards the nucleus it is noteworthy that this increased tendency to bind acid dye groups is especially marked in the chromocentre areas. This corresponds very well with the results obtained with the other cytochemical methods of investigation. The fact that the electric irritation leads to an increase of the basic protein substances in the nucleus and nucleolus in particular points to an intense irritation of the nucleolar apparatus.

**Capacity for binding basic dyes.** The fact that electric irritation leads to a distinct increase in the content of nucleotides affords a favourable opportunity for correlating the capacity of the cell for binding basic dyes with the content of nucleotides. Figs. 45 and 46 respectively show in outline preparations from non-irritated and electrically irritated spinal ganglion cells from a rabbit, stained with toluidine blue under standard conditions (see Chapter II Part I p. 23).

Measurements in the cytoplasm of a series of non-irritated cells of the large type gave on an average an extinction coefficient of 0.20. The corresponding value for the electrically irritated cells of the same type was 0.45. These tests thus show that the capacity for binding basic dyes increases parallel with the increase in the content of nucleotides in the cytoplasm.

## 2. Irritation up to 10 Minutes.

The ultraviolet cytological picture of spinal ganglion cells after irritation up to 10 minutes is entirely unlike the picture after irritation for 5 minutes only. Most of the cells in the ganglion show the same feature. An example of such a cell, photographed at  $2570\text{ \AA}$ , is given in Fig. 47. The nucleus as a whole absorbs very intensely, and no nucleolus is observable in the uniformly dark nucleus of the ultraviolet photograph. The absorptive capacity of the cytoplasm, on the other hand, is low, compared with that of the nucleus and of the spinal ganglion cells irritated for 5 minutes only. The absorption spectra show for the nuclear substance a moderate content of nucleotides and proteins in high concentrations. The tyrosin maximum lies at  $2900\text{ \AA}$ , and the capacity for binding acid dye groups is very high, indicating the markedly basic character of the proteins.

The Feulgen reaction gave the same picture as that described in regard to ganglion cells from animals at rest (see Chapter I, Part II p. 67), thus a scanty amount of Feulgen-positive particles which, on a visual estimate, did not correspond to the amount of nucleotides obtained in the above reported absorption measurements. This indicates that the nucleotides of the nucleus are mainly of ribose type.

As pointed out above, the nucleolus is not visible in the large concentrations of proteins and ribose nucleotides in the nuclear substance of these cells. On staining with cyananthrol, however, a distinct nucleolar structure is brought out, which does not differ in form from that of the nucleoli of the non-irritated cells; and the Feulgen reaction shows the previously described picture of a number of elements rich in ribose nucleotides, perinucleolarly arranged.

The cytoplasm in these cells is poor in ribose nucleotides as well as in proteins, which latter showed merely a slight tendency to bind acid dyes. No diminution in the size of the cells, as compared with that of cells from non-irritated ganglia, could be observed.

*Inhibition tests.* The above reported experiments show that, when a spinal ganglion is irritated with an electric current for up to five minutes, a process is started in the nerve cells, which leads to the

formation of proteins rich in hexone bases and ribose nucleotides both in nucleus and cytoplasm. In order to obtain some idea as to what cellular system is involved in this process, repeated attempts were made to inhibit its course. For this purpose substances such as cyanides and urethane, which tend to check certain of the oxidative cell processes, were employed. In these simple tests 8 young rabbits (including four for control) were employed, and altogether 10 spinal ganglia were analyzed in accordance with the procedure described in Chapter I Part II, p. 60). For purposes of control, the ganglion on the opposite side in the same segment was extirpated and was laid in a 37° Ringer's solution for some minutes, whereupon it was irritated for the same length of time as the exposed ganglion used for the actual test.

With a view to ascertaining the content in the ganglion cells of substance with peroxydase effects, Nadi-reaction was performed on the extirpated and cleft spinal ganglia. The one half of the ganglion was examined under a dissecting microscope, whilst the other half was fixed with a formalin sublimate solution and was sliced after embedding in paraffin. The reaction was performed firstly after addition of the Nadi reagent solely, and secondly after the addition of KCN and H<sub>2</sub>O<sub>2</sub>.

On the addition of the Nadi reagent solely, an intense bluish purple staining of the ganglion cells — which was also verified in the magnified preparations —, was obtained after a few minutes on the surface of the section. After the addition of catalase (prepared according to AGNER 1941) no corresponding stain was obtained in the cell layer of the section, though after an hour a faint violet tinge could be observed, especially on the surface. In the presence of potassium cyanide the ganglion remained entirely unstained. These tests indicate the existence of substances with peroxydase effects in the ganglion cells.

In the inhibition tests the pneumogastric nerve, during operation on either side of *Gangl. nodosum*, was infiltrated with a 1 % neutral solution of potassium cyanide. After the lapse of half an hour the ganglion was irritated for five minutes. The result was a distinct increase in the content of ribose nucleotides in the cells. In the control test, however, no increase in the nucleotide content was obtained.

### 3. Survey of Experiments with Electrical Irritation of Spinal Ganglion Cells.

According to previous cytological investigations, an increase in size is obtained on electric irritation of ganglion cells. LUGARO (1895) showed such an increase as regards the nucleolus and cell-body and, in a lesser degree, the nucleus. Concurrently with this change in size, he considered that an increase in the amount

of chromophil substance could be established. After protracted irritation a decrease in the size and stainability of the cell could be observed.

The cytochemical investigations showed, after *electric irritation for 5 minutes*, an increase in the cell's content of nucleotides and proteins both in the nucleus and in the cytoplasm. Figs. 48 and 49 respectively show an outline picture in ultraviolet at 2570 Å of non-irritated spinal ganglion cells from a rabbit and of such cells electrically irritated for 5 minutes.

Ribose nucleotides could be shown in the *nucleus* in all the types of irritated ganglion cells. In the non-irritated cells nucleotides of that character were observed only in cells of the small type. The protein content in the nuclei of the irritated cells showed a considerable increase. Measurements of the capacity for binding dyes indicated that the amount of basic groups in the protein substance was very considerable, especially in the nucleoli and chromocentre areas.

In the cytoplasm of the irritated cells an increase of ribose nucleotides amounting to 3—6 times the content in the non-irritated cells could be observed.

This cell picture thus represents a cytoplasmic protein-forming system stimulated to intense activity. A detail of special interest is that the increase of the proteins rich in hexone bases is particularly marked in the chromocentre areas, which well corresponds with the above view that the heterochromatin is the primary source of production of such proteins.

Among the cells irritated *for 5 minutes* one could actually observe some with a peripherally situated nucleus, flattened or irregularly delimited towards the centre of the cell. In the cytoplasm outside this area ribose nucleotides in large concentrations could be shown. In appearance and chemical composition these cells correspond well with the previously mentioned ganglion cells of certain fishes (see Chapter I, Part II p. 70). These cells must be regarded as extreme forms with a maximum activity of the cytoplasmic protein-forming system. Such cells could be shown in the electrically irritated ganglia, but are very unusual in the non-irritated.

After electric irritation *up to 10 minutes* a marked increase in the content of ribose nucleotides and proteins could be observed in the cytoplasm, whilst the nucleus contained proteins rich in hexone bases and ribose nucleotides in large concentrations.

Attempts to inhibit the formation of nucleotides during electrical irritation by the use of potassium cyanide and urethane, led to no result.

### Summary, Chapter IV.

Experiments were made with electric irritation. They show that after five minutes' irritation with faradic current, the cytoplasmic protein-forming system is engaged in intense productive activity. After longer irritation the cytoplasm is steadily drained of proteins and nucleotides. The probable explanation — in good analogy with the above reported experiments on motor cells —, is that the steady consumption of the proteins in the cell during the prolonged irritation is followed by the formation of new protein, which, however, owing to the physiologically violent effects of the protracted faradic treatment, is unable to replace the disintegration.

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## CHAPTER V.

### Nucleotide and Protein Metabolism in the Nerve Cell after Excision of the Axon.

The material was taken from 27 rabbits and consisted in ganglion cells of *Nucleus hypoglossi*, *Gangl. nodos.* *N. X.* as well as spinal ganglion cells of *N. ischiadicus*. A table showing the time which had elapsed before the animals were killed after the operation is subjoined. The number of rabbits used in each experiment is given in brackets.

Time	Number of rabbits
19 hours . . . . .	(3)
3 days . . . . .	(6)
8 days . . . . .	(4)
15 days . . . . .	(7)
28 days . . . . .	(2)
33 days . . . . .	(3)
150 days . . . . .	(2)

The material was fixed in accordance with Gersh's freezing-drying method or in Carnoy's solution. For purpose of control, corresponding material from non-operated rabbits as well as ganglion cells of the contralateral nucleus or ganglion were employed.

As pointed out in the above review of the literature, according to VAN GEMERTEN and others, there is a distinct difference in the reaction of the motor and the sensory nerve cells after excision of the nerve, in that the sensory cells 15 days after the injury do not pass into a recovery phase, but degenerate and are phagocytized. The author could partly confirm these observations, having found in two cases 33 days after the excision of the vagus nerve that the number of cells in *Gangl. nodosum* has greatly diminished

and that the ganglion cells had shrunk and were difficult to fix, whence no analysis with the methods here adopted could be made. In the third case in this particular test, the author found in the said ganglion that most of the cells, on cytochemical examination, gave the same result as the other types of cell examined after the lapse of 15 days or more from the excision. A few cells from *Gangl. nodosum*, however, showed the recovery pictures described below.

In the other tests uniform results were obtained with motor and sensory nerve cells examined shortly after the excision of the nerve and a considerable time later. Examples are given of the results of these studies on (1) spinal ganglion cells and cells from *Gangl. nodosum* examined 19 hours to 15 days after nerve excision and (2) cells of the first-mentioned type examined 15 to 150 days after the excision.

The absorption spectra and the photographs reproduced below are from cells of *Gangl. nodosum* and spinal ganglia.

The cells were analyzed in accordance with the procedure described in Chapter I, Part II, p. 60. Altogether 26 series were arranged in which complete absorption spectra were taken, as well as about 20 in which the rough method for determining the distribution of nucleotides which is afforded by ultraviolet microscopy under uniform conditions (see Chapter II, Part I) in addition to the other methods, was employed.

## 1. Distribution of Nucleotides and Proteins in the Normal Ganglion Cell.

**Ultraviolet microscopy.** An uninjured cell from *Gangl. nodosum N. X.*, photographed at 2570 Å, is shown in Fig. 50. The nucleus is round and centrally situated; it contains an intensely absorbing nucleolus, whilst the remainder of the nuclear substance shows a rather low absorptive power. In the cytoplasm we observe large accumulations of intensely absorbing substance.

**Absorption measurements.** A group of absorption spectra are shown in Fig. 51. Curve 1 is taken at a point in the nuclear substance between the nucleolus and the nuclear membrane. At 2900 Å we see a faint protein band with the tyrosin maximum shifted towards long wave-lengths, indicating that the protein is rich in hexone bases. In regard to the capacity for binding acid dyes, see below. No nucleic acid band can be observed.

Curve 2 is taken at a point in the intensely absorbing cytoplasm in the same cell, whilst curve 3 is from a similar cell in the same section. At 2600 Å a distinct nucleic acid band and at 2800 Å a marked protein band are observed. The tyrosin maximum lies at 2900 Å. An analysis of the absorption spectra showed on an

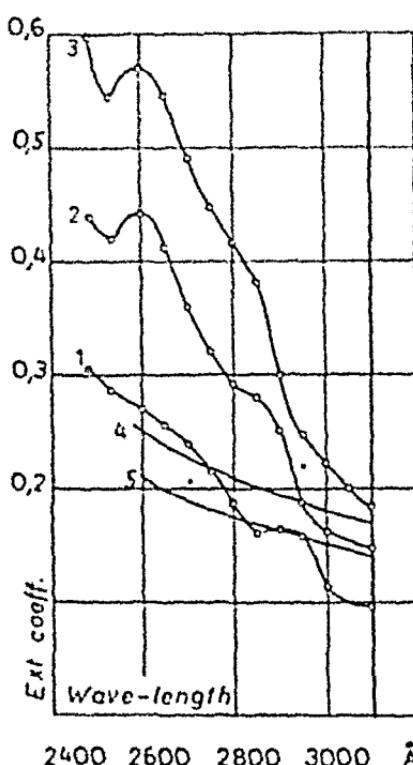


Fig. 51. Absorption spectra from points in the nucleus (curve 1) and cytoplasm (curve 2) in an uninjured spinal ganglion cell from *Gangl. nodos. N. X.* from a rabbit. Curve 3 is taken in the cytoplasm of another similar cell. The curves for the cytoplasm show a marked nucleotide band and protein band with a tyrosin maximum shifted towards long wave-lengths. Curves 4 and 5 indicate the loss of light by scattering for curves 2 and 3, respectively.

average 36--38 per cent of protein and 2.4--2.8 per cent of nucleotides. As regards the capacity for binding acid dyes see below.

The Feulgen reaction gave a negative result for the nucleolus and for the large concentrations of nucleotides in the cytoplasm, which shows that they are of ribose type.

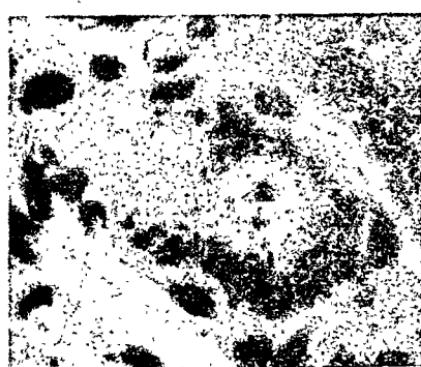
**Micro-Incineration.** A group of incinerated cells, photographed in the dark field, is shown in Fig. 52. Corresponding to the site of the nucleolus and the parts of the cytoplasm absorbing intensely at 2600 Å, large amounts of ash are observed.



Fig. 50. Nerve cell of *Gangl. nodos. N. X.* from uninjured rabbit. Carnoy-fixed. Large amounts of ribose nucleotides in the cytoplasm. Magnification 1,150 $\times$ . Objective aperture 0.85. Condenser aperture 0.6.



Fig. 52. Micro-incinerated nerve cells of *Gangl. nodos. N. X.* from uninjured rabbit in the dark field. At the place corresponding to the accumulations of cytoplasmic nucleotides, absorbing at 2600 Å, large residue of ash. Magnification 700 $\times$ .



Figs. 53 and 54. Nerve cells of *Gangl. nodos. N. X.* from rabbit, 19 hours after excision of axon. Carnoy-fixed. Disappearance of ribose nucleotides within a sectorlike area in the cytoplasm. Magnification and optics as in Fig. 50.

HYDÉN: Protein metabolism in the nerve cell.



Fig. 56. Nerve cells of *Gangl. nodos. N. X.* from rabbit, 3 days after excision of axon. Carnoy-fixed. Close to the eccentrically situated nucleus well-developed nuclear-membrane nucleotides. Remainder of cytoplasm poor in nucleotides. Magnification and optics as in Fig. 50.

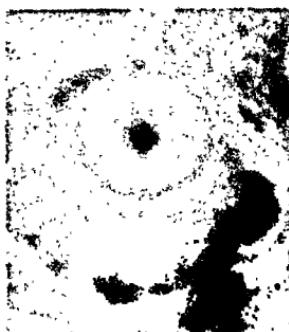


Fig. 57. Another ganglion cell from same section. Cytoplasm devoid of nucleotides. Magnification and optics as in Fig. 50.

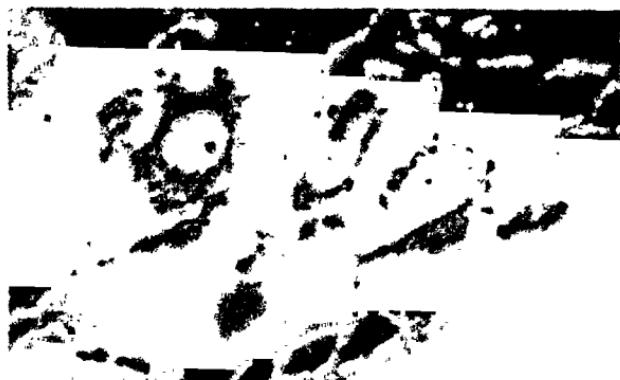


Fig. 58. A group of micro-incinerated nerve cells of *Gangl. nodos. N. X.* from rabbit, 3 days after excision of axon, in the dark field. Merely small amounts of ash in the cytoplasm. Magnification 700 X.

## 2. Changes in the Ganglion Cell 19 Hours after Nerve Excision.

**Ultraviolet microscopy.** Most of the cells in the ganglion show the same ultraviolet picture as the cells of an uninjured ganglion. In a few of them, however, certain changes can be observed. Examples of such cells are given in Figs. 53 and 54. The nuclei in these cells are situated somewhat excentrically. The accumulations of absorbing substance are no longer sharply delimited, being rather diffuse. In the cytoplasm there is a sector-like area with a considerably lower absorptive capacity than the surrounding parts.

**Absorption measurements.** Curve 1 in Fig. 55 is taken at a point in the nuclear substance between nucleolus and nuclear membrane.

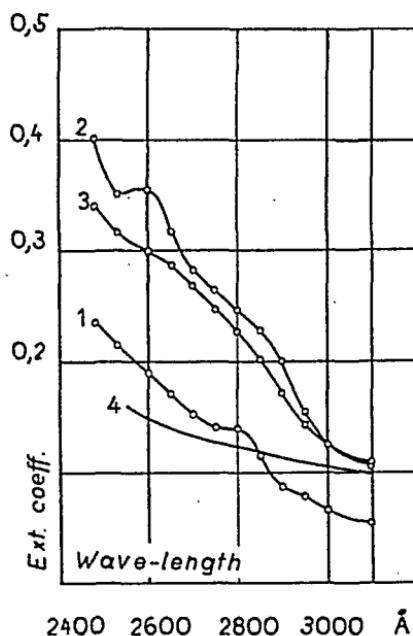


Fig. 55. Absorption spectra from points in a cell from *Gangl. nodos. N. X.* 19 hours after excision of the axon. Curve 1 is from a point in the nucleus, curve 2 from a point in the intensely absorbing part of the cytoplasm, curve 3 from the sector-like area in the cytoplasm with a smaller absorptive power (See Fig. 54). The last-mentioned curve is devoid of a perceptible nucleotide band and has merely a faint protein band. Curve 4 indicates the loss of light for curves 2 and 3.

A nucleotide band is missing, but at 2800 we see a faint protein band with the tyrosin maximum lying at 2850 Å, which indicates the occurrence of proteins rich in hexone bases in small concentrations.

Curve 2 is taken at a point in a peripherally situated, intensely absorbing part of the cytoplasm. The curve is in complete correspondence with that taken in the cytoplasm of uninjured cells.

Curve 3 is taken at a point in the sector-like part of the cytoplasm. No nucleic acid band can be seen: the curve shows merely a faint protein band.

Curve analysis of the absorption spectra showed that the nucleic acid concentration in the changed area was merely one-third as high as in the nucleotide-containing parts of the cytoplasm, with the ultraviolet cytology of the uninjured cell, and the protein concentration was merely half as high.

A marked decrease in the content of nucleotides and proteins within a certain part of the cytoplasm can thus be observed in a few cells as early as 19 hours after nerve excision. Cytologically, the changed cytoplasmic area shows the picture of chromatolysis.

### 3. Changes in the Ganglion Cell 3 Days after Nerve Excision.

**Ultraviolet microscopy.** All the cells in the ganglion are markedly changed. On photographing at 2570 Å, two different types of cells with different ultraviolet cytological pictures can be distinguished.

An example of one of the types is given in Fig. 56. The nucleus is situated near the periphery of the cell. Outside the part facing the centre of the cell large accumulations of highly absorbing substance can be observed. The remainder of the cytoplasm is but slightly absorbing.

An example of the second type of modified cells is given in Fig. 57. There are no accumulations of absorbing substance close to the nucleus. The entire cytoplasm is marked by a low absorptive capacity.

**Absorption measurements.** The accumulations of absorbing substance close to the nucleus were found to contain large concentrations of ribose nucleotides as well as proteins, which were shown by the staining tests to be rich in basic groups (see below). In location and composition the absorbing substance close to the nucleus thus shows striking similarities to the nuclear-membrane nucleotides in the ganglion cells of *Lophius piscatorius* and in

the electrically irritated cells. Also from a purely cytological point of view, there are great resemblances between these cells.

The absorption measurements from the cytoplasm in ganglion cells after 3 days showed a further decrease in the content of protein substance. Examples of two of the absorption spectra

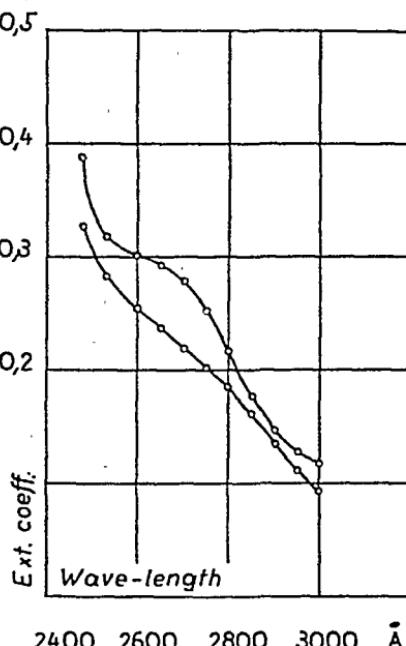


Fig. 58. A couple of absorption curves from points in the cytoplasm of cells of *Gangl. nodos.* N. X., from a rabbit, 3 days after excision of the axon. The curves show merely faint protein bands.

taken a points in the cytoplasm of such cells are given in Fig. 58. The curves are flat and show merely faint protein bands at 2570 Å.

**Micro-incineration.** Some micro-incinerated cells, photographed in the dark field, are shown in Fig. 59. The ash residue in the cells is very scanty and, in comparison with that of the uninjured cells (see Fig. 52), the difference is very considerable.

#### 4. Changes in the Ganglion Cell 15 Days after Nerve Excision.

The curves have a very small absorptive capacity at 2570 Å, and almost all of them show the same cytological picture with the very excentrically situated nuclei. The absorption measurements merely show the occurrence of proteins in small concentrations. No nucleotide band is observed. As indicated in

the review of the literature, SPATZ and other authors contend that the process in retrograde reaction is mainly characterized by the taking-up of fluids by the cell, giving rise to the typical picture of chromatolysis and the peripheral displacement of the nucleus. The above reported results of the author's tests, which showed a decrease in the cell content of nucleotides and proteins, might likewise conceivably be attributed to an increase in volume owing to the absorption of fluids. An examination of the average absorption measurement figures show, however that this is not the case. The actual absorption at 2600 Å, measured in the cytoplasm of the control ganglion cells averaged  $\epsilon = 0.24$ , and in the cytoplasm of cells examined 15 days after nerve excision averaged  $\epsilon = 0.09$ . Had this disparity been due to an increase in volume, the original maximum cross-sectional area would have increased to  $\left(\frac{0.24}{0.09}\right)^{\frac{1}{2}}$ , i. e. to 193 per cent. Disparities of anything like this magnitude could not be observed on comparison with the cell sections comprising the major part of the nucleolus in longitudinally sliced ganglia from the excised nerve and the contralateral nerve.

### 5. Spinal Ganglion Cells 28, 33 and 150 Days after Nerve Excision.

The examples adduced are taken from the spinal ganglions of *N. ischiadicus* in rabbits. Most of the cells in ganglia examined after the lapse of 28 and 33 days still show the same ultraviolet picture as in the 15-days' ganglion, with excentrically situated nuclei and but slightly absorbing cytoplasms. In some cells, however, the nuclei are situated more centrally. Close to the nucleus in this type of cells an area with higher absorption is seen in the cytoplasm.

In the spinal ganglia examined 150 days after the nerve excision, about one-third of the cells show the most shifting ultraviolet cytological pictures. The outline views show a striking resemblance to ganglia from *Lophius piscatorius*.

A type of cell with nucleotides in a grouping similar to that observed in ganglion cells a few days after the nerve excision is found in Fig. 60. The nucleus is situated at the periphery of the cell, being flattened and irregular delimited towards the centre. Close to this part of the nucleus, accumulations of highly

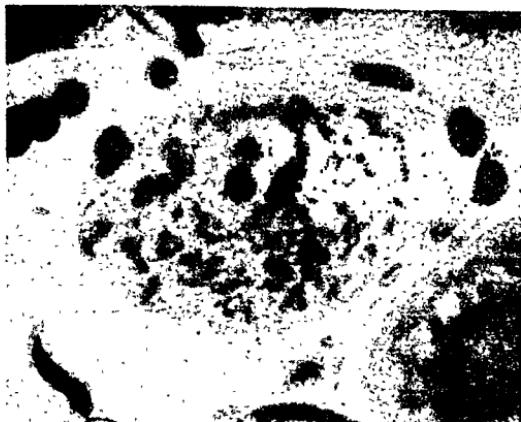


Fig. 60. Spinal ganglion cell, rabbit, 150 days after excision of axon. Carnoy-fixed. Nucleus excentrically situated, close to it nuclear-membrane nucleotides. Magnification 1,150  $\times$ . Objective aperture 0.85. Condenser aperture 0.6.



Fig. 61 and 62. Spinal ganglion cells, rabbit, 150 days after excision of axon. Nucleus centrally situated. Close to it immense concentrations of nuclear-membrane nucleotides. Magnification and optics as in Fig. 60.

HYLTÉN: Protein metabolism in the nerve cell.



Fig. 63. Spinal ganglion cell 150 days after excision of axon. The ribose nucleotides in the cell restored. Differs from ultraviolet picture of uninjured spinal ganglion cells by the larger aggregations of cytoplasmic nucleotides.

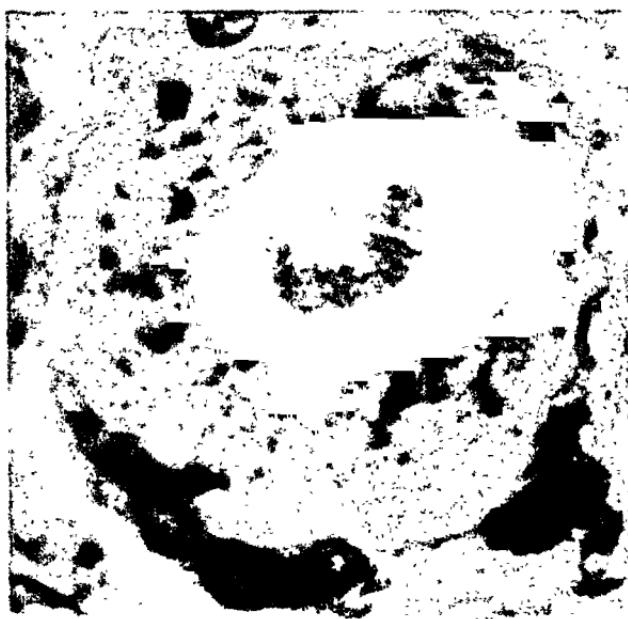


Fig. 64. Spinal ganglion cell, rabbit, 150 days after excision of axon. Stained with  $\text{FeCl}_3$  under standard conditions. The nucleolus and the areas close to the nucleus at the site of the nuclear-membrane nucleotides have bound the acid dyes with great avidity.

absorbing substance in the cytoplasm are observed. The absorption spectra showed that they contained large concentrations of ribose nucleotides, and were thus identical with the above described nuclear-membrane nucleotides.

In other cells the nucleus is more centrally situated (see Figs. 61—62.) Particularly striking are the well-developed areas with highly absorbing substance, which completely surround the nucleus, and which were found to contain very high concentrations of ribose nucleotides. In regard to their tendency to bind acid dyes, see below. They consist in nuclear-membrane nucleotides, developed to such an extent as had not been observed in any previous test.

As above mentioned, the great majority of the cells in the 150-days' ganglia showed the ultraviolet cytological picture illustrated by the cell in Fig. 63. The nucleus, which is centrally situated, is spherical, and the cell-body shows the same form as in the cells of an uninjured ganglion. The tests with absorption measurements and with staining likewise showed a distribution and content of ribose nucleotides and proteins completely corresponding to that of the cells in an uninjured ganglion. Collections of ribose nucleotides in the cytoplasm are brought out on the photograph. A replacement of the content of nucleotides and protein substance in the cell must thus have occurred.

**Capacity for binding acid dyes.** Measurements of ganglion cells in an uninjured ganglion, stained with cyananthrol in accordance with the technic described in Chapter II, Part I, p. 21, showed an average extinction coefficient of 0.17 for the nucleus and of 0.26 for the cytoplasm. For purpose of comparison, cells from a ganglion examined 5 months after excision of the axon were selected, and their uniform staining was checked by computations of the erythrocytes. A cell of this nature, photographed at 5750 Å, is shown in Fig. 64. In the nucleus we see the highly stained nucleolus, and round the nuclear membrane intensely stained areas are observed, whereas the rest of the cytoplasm is faintly stained. Measurements showed an average extinction coefficient of 0.22 for the nucleus, of 0.40 for the intensely stained area corresponding to the site of the nuclear-membrane nucleotides, and of 0.12 for the rest of the cytoplasm, where the absorption measurements showed a decrease in the protein content. The results of these staining tests completely correspond with the absorption-measurement data, and show the reaction of the basic proteins to the

irritation of the protein-forming system which can be observed with other cytochemical methods during the recovery processes in the cell after excision of the axon. The content of basic proteins increases in the nucleus and is particularly high at the spot where the newly formed nuclear-membrane nucleotides in the cytoplasm are located. In the remaining part of the cytoplasm it is low, which is in correspondence with the absorption measurements.

Capacity for binding basic dyes. The measurements after staining with toluidine blue, in accordance with the procedure described in Chapter II, Part I, p. 23, showed in the cytoplasm of the uninjured ganglion cells of *Gangl. nodos. N. X.* an average extinction coefficient of 0.35. In cells examined 8 days after the excision of the nerve the extinction coefficient averaged 0.13.

These tests, like those reported in Chapter IV, Part II, p. 93, show that the capacity for binding basic dyes varies with the content of nucleotides in the cells.

## 6. Electrical Irritation of Ganglion Cells with a Reduced Content of Nucleotides and Proteins after Nerve Excision.

Electrical irritation of an "uninjured" ganglion for 5 minutes resulted in a marked increase of the nucleotides in the cells. It was, therefore, of interest to ascertain whether this result could be produced also in ganglion cells in which the nucleotide and protein content had been greatly reduced after nerve excision.

For this purpose the *Gangl. nodosum* of a rabbit, 3 and 8 days, respectively, after excision of the vagus nerve, was irritated with 1 MA for 5 minutes. For purpose of comparison, a ganglion which had already been thus treated was laid in Ringer's solution for 10 minutes, whereupon it was irritated in the same way.

On examination, no increase in the content of nucleotides in the ganglion cells could be observed.

## 7. The Retrograde Reaction as a Transitory Inhibition of the Protein-producing System in the Ganglion Cell.

Excision of the axon was found to result in considerable changes in the chemical composition of the ganglion cell in question. Even after the lapse of only 19 hours a decrease in the content

of ribose nucleotides and proteins within a sector-like part of the cytoplasm and some displacement of the nucleus towards the periphery could be noticed. After 3 days the said decrease had been extended to the greater part of the cytoplasm; on the other hand, the production of nuclear-membrane nucleotides could be observed in some of the cells. After 15 days the main content of the cytoplasm consisted in merely small amounts of protein substance. Distinct concentrations of nucleotides were completely lacking. The cells now presented the cytological picture of a pronounced chromatolysis.

This cytological picture results from a marked decrease in the content of nucleotides and proteins.

After the lapse of 28—33 days an incipient replacement of the nucleotides and proteins in the cell could be noticed. The first indication of this was the production of nucleotides at the nuclear membrane. It was possible to follow their development whilst the cytological picture of an "uninjured" cell in ganglia examined 150 days after nerve excision was being recovered. The nucleotides of the nuclear membrane were at first located solely in the part of the peripherally displaced nucleus facing the centre of the cell. In cells with more centrally situated nuclei the nucleotides of the nuclear membrane attained their greatest development, encompassing the entire nucleus.

The production and development of the nuclear-membrane nucleotides can be observed in the ganglion cell immediately after the nerve excision, when the content of protein substance and nucleotides in the cell is rapidly diminished, as well as during the recovery stage, when, after the lapse of some time, those substances increase so that the original content is eventually restored. It has been pointed out above, in regard to other ganglion-cell material, that these nucleotides participate in the formation of cytoplasmic protein, and that their production must be viewed as an indication of an intense activity of the protein metabolism system in the nerve cell.

As previously indicated, these periods of breaking-down and restoration, after nerve excision, of substances in the cell begin with the production of nucleotides in the nuclear membrane. During the intervening period the content of protein substances in the cell is very small. The ganglion cell then, so to speak, "lies fallow" in regard to the endocellular nucleotide and protein metabolism. This statement is borne out by the fact that it is

not possible, as it was in the case of "uninjured" ganglion cells, to increase the content of nucleotides and proteins by electrical irritation. On the other hand, it has been found that the increase of cellular substance, resulting from the irritation of "uninjured" cells, is accompanied by all the signs of an intense activity of the nucleolar and protein-producing system.

The above-mentioned changes in the chemical composition of the ganglion cell after nerve excision show that *the retrograde reaction has the character of a transitory inhibition of the protein-producing system in the ganglion cell.*

During the busy processes of metabolism which accompany the recovery of the ganglion cell, new axons develop at the periphery, pass across the wound, and the peripheral nerve-end is regenerated. As CAJAL (1928) and other investigators have established, the growth of the new axons begins at an early date, namely one or two days after excision of the nerve. According to observations made by the said author on rats, the rate of growth is very rapid, amounting to 2.5—4 mm a day. He found that, after a week, the axon had passed completely across the wound, and other authors (e. g. REXED and SWENSSON, 1941) state that myelin sheaths can be observed after the lapse of another week. The Schwann's cells in the wound show every sign of an intense activity of the nucleolar and protein-forming system (HYDÉN and REXED, 1943). A large nucleolus, rich in ribose nucleotides, appears and the nucleus is expanded to about fifty times its original size. These changes in the Schwann's cells proceed during the course of one or two weeks. After that time the big nucleolus vanishes, and the size of the nucleus is much the same as in the Schwann's cells of an uninjured nerve-fibre. Although the changes in the nucleotide and protein metabolism in the ganglion cells as well as in the Schwann's cells were induced by the same operation, namely the excision of the nerve, there is no synchronism between the central and the peripheral reaction which affects the ganglion cell and the neurilemma, respectively.

On the other hand, the short space of time during which the Schwann's cells pass through their independent cell-cycle, with endocellular increase of the protein substance, is synchronous with the time required for the new axons to pass across the wound and to be myelinated.

As for the restoration of function, observations on rats (HOBORUT and JALOWY, 1936) have shown that the chronaxie in the nerves

attains normal values 28 days after excision of the sciatic nerve. After the lapse of that time it may thus be expected that the motor neuron will have resumed its function.

The above reviewed investigations with cytochemical methods showed an increase in the content of nucleotides and proteins in the ganglion cells after the lapse of about a month. *This indicates the close connection of the nucleotide and protein metabolism processes with the function.*

These cytochemical observations, taken together, reflect a continuous physiological process, where the several types of cell, after varying times, represent different phases in the protein-forming process. The chronological order may be diagrammatically represented as follows. See the chart, Fig. 65, where the

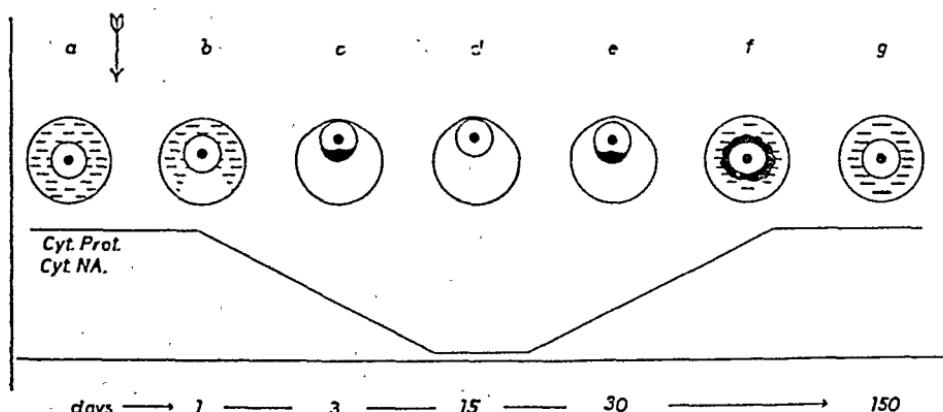


Fig. 65. Chart illustrating the extensive protein-forming processes after excision of the axon. (See the text.) The ordinate indicates the concentration of nucleotides and proteins in the ganglion cell cytoplasm. The abscissa shows the lapse of time after excision of the axon (marked by an arrow). The horizontal lines and dark areas indicate ribose nucleotides.

ordinate indicates the content of nucleotides and protein substance in the cell, and the abscissa shows the time. The letter *a* denotes a normal ganglion cell with large concentrations of ribose nucleotides and proteins in the cytoplasm. Scarcely 24 hours after excision of the axon (marked by an arrow), the content of nucleotides and proteins begins to diminish within a limited part, whilst the nucleus is displaced towards the periphery (*b* in the chart). The diminution proceeds rapidly, and a few days after the injury it has reached a considerable extent. Concurrently with this rapidly advancing process, large concentrations of nuclear-membrane nucleotides are formed in some cells close to the peripherally situated nucleus (*c* in the chart). This may be

interpreted as an attempt on the part of the cell, by intense activity of the nucleolar and protein-forming system, to compensate the disturbance of the protein metabolism caused by the injury to the axon, or to assist in the rapid increase of the cell substance which is proceeding at the periphery during the growth of the axon. After a fortnight or so the principal content of the cell consists in small concentrations of proteins mainly with the character of structural substance (*d* in the chart). The cell "lies fallow", and for a short space of time the protein-forming system in the ganglion cell is completely inhibited. When this inhibition has continued for several days, a restoration of the nucleotides and proteins in the ganglion cell commences.

This restoration is effected by an intensive functioning of the protein-producing system in the cell. The first indication of this process is the production of nuclear-membrane nucleotides (*e* in the chart) close to a local sector of the nucleus. During the following phase the nucleus gradually resumes its central position (*f* in the chart). The nuclear-membrane nucleotides surround the entire nucleus, and are amassed in immense concentrations. From the compact layer close to the nucleus the nucleotides thin out towards the periphery of the cell, where they have the characteristic appearance of small, limited aggregations.

After the lapse of 150 days most of the cells show the ultra-violet cytological picture of the uninjured ganglion cell (*g* in the chart). The only difference is that the aggregations of ribose nucleotides in the cytoplasm are larger.

### S. Recapitulation.

After excision of the axon, processes of protein metabolism are busily at work in the cell. During the next few days after, the excision the content of nucleotides and proteins is reduced, whilst the protein-forming system shows signs of intense irritation, and nuclear-membrane nucleotides appear in large amounts. After a fortnight or so the cell has been drained of most of the nucleotides and proteins, the pictures of irritation vanish, and the protein-forming system is inhibited; this is indicated by the fact that electric irritation fails to induce the formation of nucleotides and proteins. After the lapse of another fortnight the content of proteins in the cell begins to be restored, and at the same

time one notes the appearance of nuclear-membrane nucleotides which are so well-developed that no parallel in any other mammalian material can be shown. According to statements in the literature, these processes are synchronous with the restoration of function in the motor neuron.

## 9. Earlier Macro-chemical Studies Indicating the Occurrence of Protein Metabolism Processes in the Central Nervous System.

It has been shown above that after motorial exhaustion and excision of the axon the content of nucleotides and proteins in individual ganglion cells is greatly reduced. The differences in content are in the ratio of about three to one.

The ganglion cell itself, in volume, is but an insignificant part of the neuron. In ganglia and nuclei within the central nervous system, however, these cells constitute the major part of the tissue substance. It might therefore be expected that the said endocellular changes in chemical composition would be reflected in macro-chemical investigations of individual cells and "gray matter" under similar experimental conditions.

Studies suggesting a marked protein metabolism in the gray matter under certain conditions can in fact be found in the literature. Below follows a short review of some of the most representative investigations. See further reviews by e. g. WINTERSTEIN 1929, QUASTEL 1939.

The differences in composition between gray and white matter are very considerable. The water content in gray matter amounts to 84 %, in white to 70 % (ABDERHALDEN and WEIL, 1910). According to KOCK and KOCH (cited by SCHMITZ), the solids in gray matter amount to 16.83 %, in white to 30.30 %. The protein content in gray matter amounts to 10.2 %, in white to 8.8 % (SCHMITZ 1929). The percentage of proteins relatively to the solids is 47.10 in gray matter, 27.1 in white. According to values computed by BODANSKY (1934), gray matter contains 8 % proteins, of which 3 % consists of nucleoproteins. In white matter the proteins amount to 7 %, including 3.7 % nucleoproteins.

*Macro-chemical investigations of gray matter after motorial exhaustion and intoxication with cramp-producing poisons.* SOULA

and FABRE (1913) studied the effects of muscular work by making rats run in a working-machine till complete exhaustion. On examining certain macerated part of the central nervous system — they do not give particulars of the parts dissected —, they found that the aminogenesis coefficient (amino-nitrogen in percentage of the total nitrogen) and proteolysis coefficient (polypeptid nitrogen plus amino-nitrogen in percentage of the total nitrogen) had been considerably raised. Cytologically, the picture of a chromatolysis in the anterior horn cells of the spinal cord could be observed. The conclusion drawn by the authors was that the basophil substance in the nerve cells might be considered to represent a nitrogenous reserve.

On similar examination of macerated parts of the central nervous system, after the administration of certain poisons, SOULA (1912--1913) found that cramp-producing poisons raised the proteolysis coefficient to double the value, whilst paralyzing poisons reduced it.

*The effects of irritation by light on the visual centres.* GORODISSKY (1926), availing himself of the irritation caused by light, studied the proteolysis in the visual centres of cats, some of which for a time had had their eyelids sewn up. As an indication of the proteolysis, he adopted the residual nitrogen in percentage of the total nitrogen. Removal of the irritation resulted in the slowing-down of the proteolysis in the visual centres as compared with seeing animals, but not in other parts of the brain. After resumption of the irritation, the proteolysis value rose. The same effects were observed in kittens, whose eyelids were sewn up before they had been opened. The proteolysis value for adult seeing animals was nearly three times as high. As pointed out above (p. 75), concurrent data regarding chromatolysis in the ganglion cells of the retina are found in the literature.

*Electrical irritation.* SOULA (1912) studied the effects of electrical irritation on the intensity of the proteolysis in the "gray matter" of rabbits. The animals were irritated with 3 MA. The result was an increase in the amino-acid genesis by practically double. As it was also conceivable that the increase in the amino-acid nitrogen was derived from the tetanized muscular tissue, that author investigated the matter, but could not observe any increase of this value in such muscles. An increase of the aminogenesis coefficient in the gray matter was found also on electrical

irritation of intensely curarized animals. The conclusion drawn by SOULA from these tests was that the activity of the brain centres is associated with a consumption of nitrogenous substances.

HIRSCHBERG and WINTERSTEIN (1918) studied the nitrogen metabolism in the spinal cord of frogs after electrical irritation, and found that it had increased by more than three times the "resting" value. In order to obtain an insight into the nature of the nitrogenous substances, the said authors studied the formation of ammonia after electrical irritation of the spinal cord of frogs. "In rest" the nitrogen given off to the RINGER's solution in which the cord had been laid consisted mainly of nitrogen titratable in formalin (28—29 %), besides a small percentage of ammonia and nitrogen from lipoids soluble in water. As to about 25 % it consisted of nitrogen of unknown origin. On electric irritation the titratable nitrogen as well as the nitrogen of unknown origin increased many times over, whereas the other nitrogen fractions remained unchanged.

The administration of glucose results in an immense reduction of the nitrogen excretion, especially during electrical irritation. During that irritation the ammonium nitrate showed an increase. The residual nitrogen consisted especially in the nitrogen titratable in formalin and in the nitrogen of unknown origin.

*Nerve excision.* MARINESCO (1925) (cited by WINTERSTEIN 1929) studied the nitrogen metabolism in the spinal cord of dogs in accordance with the method of SOULA, after unilateral removal of the sciatic nerve and the brachial plexus. He then observed a distinct increase in the aminogenesis and proteolysis values.

BENKOVITCH (1939), after amputating the extremities of one side in dogs, observed a decrease of the protein decomposition products in the cortical regions concerned.

### Summary, Chapter V.

The above reviewed experimental investigations, with chemical methods, in the central nervous system do not give any uniform picture of the processes involved. The methods adopted are dissimilar, the results are divergent, and in this particular field the researches are complicated by the difficulty of isolating sufficiently large amounts of gray matter; as this substance too will

contain the nuclei of nerve fibres and mantle cells in addition to the ganglion cells, no clear-cut tests can be conducted. These investigations, however, indicate the probability of extensive processes of protein metabolism in the central nervous system and, in essentials, they do not in any way conflict with the author's own results.

## CHAPTER VI.

### Nucleotide and Protein Metabolism in Purkinje's Cells.

The experiments reported in the preceding section showed that extensive processes of protein metabolism proceed in the nerve cell in connection with its function. These results, obtained on animals which had been exposed to various operative cuts and external interferences, can scarcely be applied forthwith to the normal conditions. In order to investigate this matter, the author tackled the Purkinje's cells of the cerebellum, which, in view of their size and well-known cytology, are good objects of investigation.

#### A. Literature.

VAN DURME (1901) drew attention to the occurrence of chromophil and chromophobe Purkinje's cells. He found that the chromophobe cells on an average were larger and had the peripheral part of the cell-body free from basophil substance. The chromophil cells were readily stainable and had the largest amounts of basophil substance accumulated close to a section of the nucleus. He considered that these structures consisted of chromatin and that the two kinds of cells were in different functional states. CAJAL (1897) found an accumulation of basophil substance close to the section of the nucleus facing the largest dentrite.

STÖHR (1923) examined Purkinje's cells in ultraviolet light. He found that the dark parts corresponded to the distribution of Nissl's bodies and described an accumulation of chromatine granules in the vicinity of the nuclear membrane. In the cyto-

plasm in the same section there was an accumulation of readily stainable granule, which formed a cap on the nucleus.

SAGUCHI (1930) describes chromophil and chromophobe Purkinje's cells, stating that both these types occur in different frequencies in different animals. He describes the nucleus as oval or round, often provided with a flattened or concave area, facing the apex of the cell and adjacent to the basophil cap of the nucleus. SAGUCHI divides the chromophil cells into three types, which he interprets as successive stages in a process beginning with chromophilia. In the first stage the nucleus is readily stainable, the cytoplasm feebly. In the second the nucleus and the cytoplasm are equally readily stainable, whereas in the final stage the nucleus appears to be empty and the cytoplasm is more or less intensely chromophil. In the markedly chromophil cells the author states that he has observed a dissolution of the nucleolus. In the same type of cells the previously mentioned basophil and acidophil nucleo-nephelium (see p. 48) was well-developed, and even flowed out into the cytoplasm. In SAGUCHI's opinion the cytological finds show that the Nissl's bodies in the chromophobe cells are derived from the basophil cap of the nucleus and are closely connected with the migration of the nucleo-nephelium.

EINARSSON (1933), with his staining method, examined Purkinje's cells and described cytologic pictures which he considered to show a close relation of the basophil substance to the nucleo-chromatin. In his opinion these pictures support the view that Nissl's bodies are a product of the activity of the nucleus and are formed by diffusion from the nucleus. He thus adheres to the same view as HOLMGREN (1899), SPATZ (1920) and STÖHR (1923) regarding accumulations of a basophil substance close to the nucleus in nerve cells.

LOO (1937) studied the Feulgen reaction on Purkinje's cells in rats of different ages. The nucleolus had a faint reddish violet tint and was surrounded by a number of markedly Feulgen-positive granules. He describes also Feulgen-positive granules lying in the cytoplasm. He states that with increasing age the particles round the nucleolus diminish, whereas the extra-nuclear increases. LOO concludes from these observations that in Purkinje's cells there is a diffusion of particles containing thymonucleic acid in the cytoplasm.

## B. Own Investigations.

The material was taken from 3 cats and 10 rabbits, and was fixed in accordance with Gersh's freezing-drying method or in Carnoy's solution. It was analyzed in accordance with the procedure described in Chapter I, Part II, p. 60. In 24 serial investigations complete absorption spectra were taken, and in 10 additional series the method for determining the distribution of nucleotides which is afforded by ultraviolet photography under uniform conditions (see Chapter II, Part I) as well as other previously reported methods were adopted.

**Ultraviolet microscopy.** In photographing a cross section through a convolution of the cerebellum, one is struck by the fact that different Purkinje's cells show a completely different ultraviolet cytology. Some of them show the picture illustrated in Fig. 66. The nucleus is spherical, a large intensely absorbing nucleus is seen in the centre, whilst the remainder of the nucleus contains but little substance absorbing at that wave-length. In the cytoplasm close to a sector of the nucleus, a crescent of highly absorbing substance is observed. Otherwise the cytoplasm is but slightly absorbing. The ultraviolet absorbing picture of these Purkinje's cells (in the sequel termed "type I") greatly resembles that of the ganglion cells in *Lophius piscatorius*.

If the Purkinje cell layer in the cerebellum convolution is followed in the same section in either direction, one will usually find, about a hundred  $\mu$  from type I, a cell showing the ultraviolet picture illustrated in Fig. 69. The difference in absorptive power as compared with the preceding type of cell is most remarkable. The nucleus is rounded-off and in its centre we discern the nucleolus, which, however, owing to the great absorptive capacity of the remaining nuclear substance, is brought out very badly in the picture. The cytoplasm is marked by a very high absorption. In the sequel these cells are termed "type II".

Among these Purkinje's cells in the same convolution in the section there are cells whose capacity for absorbing at 2570 Å ranges them, in ultraviolet cytological respects, between type I and II. See Fig. 72. The plates, as far as possible, were identically copied. The cell shows a moderate absorptive power. In the photograph the nucleus comes out darker than in type II, but lighter than in type I. Close to a sector of the nucleus a cres-

cent of highly absorbing substance is seen, and the absorptive power of the remaining cytoplasm is intermediate between that of type I and type II.

### Type I.

#### Absorption measurements.

Two of the absorption spectra from points in a frozen section of a cell are shown in Fig. 67.

Curve 1 is taken at a point between the nucleolus and the nuclear membrane. The absorption is low and, overlapping the non-

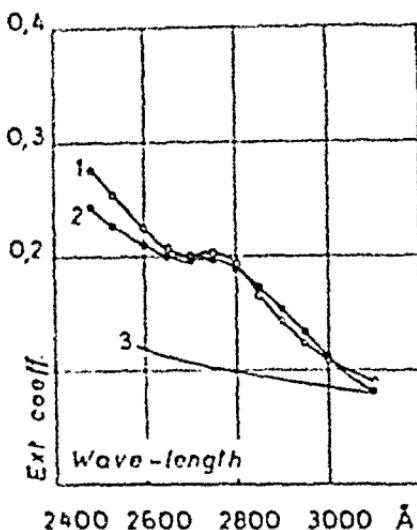


Fig. 67. Absorption spectra from points in a Purkinje cell type I. The nuclear substance (curve 1) and the cytoplasm (curve 2). Low content of proteins in the nucleus. The cytoplasm contains merely small concentrations of protein substance.

Curve 3 indicates the loss of light by scattering.

specific absorption, increasing at shorter wave-lengths, we see but a faint protein band at 2570 Å. No nucleotide band can be observed. Analysis of the absorption spectra showed an average content of 4–8 per cent of protein, whilst the nucleic acid content was below 0.1 per cent.

Curve 2 is taken at a point in the cytoplasm between the nuclear membrane and the periphery of the cell in the faintly absorbing part, and likewise shows merely a faint protein band. Analysis of the absorption spectra showed an average content of 6–10 per cent of protein, whilst the nucleic acid concentration was below 0.1 per cent. — As regards the capacity for binding acid and basic dyes, see below.

In the intensely absorbing area close to the nuclear membrane

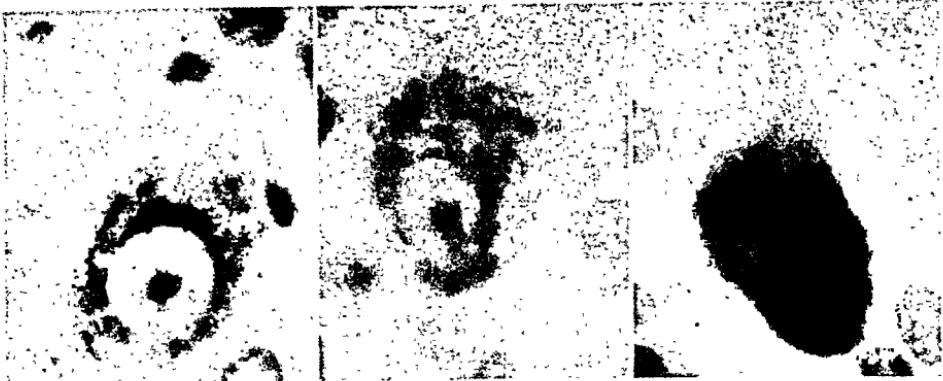


Fig. 66. Purkinje cell, type I. Cytoplasm poor in nucleotides, apart from those of the nuclear membrane. Magnification 1,150  $\times$ . Objective aperture 0.85. Condenser aperture 0.6.

Fig. 72. Purkinje cell, intermediate form. Moderate amounts of cytoplasmic and nuclear-membrane nucleotides.

Fig. 69. Purkinje cell type II. Very large amounts of ribose nucleotides in nucleus and cytoplasm.



Fig. 68. Micro-incinerated Purkinje cells of type I in the dark field. Small ash content, chiefly collected at the part of the nuclear-membrane intensely absorbing at 2600 Å. Magnification 700  $\times$ .

Fig. 71. Micro-incinerated Purkinje cells, type II, in the dark field. The whole cell yields a very large residue of ash. Magnification 700  $\times$ .

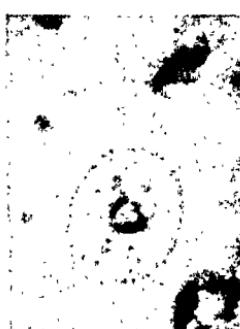


Fig. 74. Purkinje cell on which the Feulgen reaction has been performed. Nucleolus surrounded by Feulgen-positive particles. The Feulgen-positive substance in the remainder of the nucleus mainly collected in vicinity of nuclear membrane. Magnification about 1,200  $\times$ .



the measurements showed the occurrence of nucleotides in high concentrations.

**Micro-incineration.** A group of micro-incinerated Purkinje's cells of this type, photographed in the dark field, is shown in Fig. 68. At the site of the nucleolus and the nuclear-membrane nucleotides a copious residue of ash is observed, whereas the remainder of the cytoplasm is devoid of ash. This corresponds well with the absorption measurement data.

### Type II.

#### Absorption measurements.

Two of the absorption spectra are shown in Fig. 70. Curve 1 is taken at a point in the nuclear substance between the nucleolus

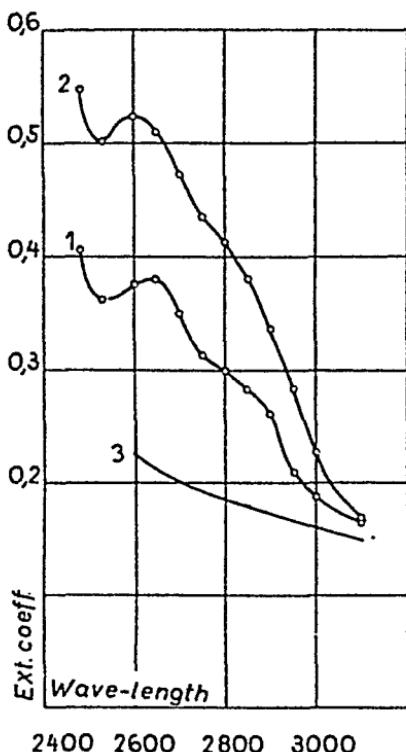


Fig. 70. Absorption spectra from points in a Purkinje cell type II. The nuclear substance (curve 1) shows a marked nucleotide band. The shape of the curve indicates the presence of proteins rich in hexone bases. Curve 2, from a point in the cytoplasm, shows large concentrations of nucleotides. Curve 3 as in Fig. 67.

and the nuclear membrane. At 2650 Å there is a distinct absorption maximum; the curve then falls towards long wave-lengths, forming a marked protein band at 2850 Å, after which it falls steeply towards 3000 Å. The tyrosin band lies at 2900 Å, which

Ich untersuchte die Einwirkung des von mir verwendeten Histamins auf die Geweboxydationen der Leber im Warburg- und Methylenblau-Versuch. Dabei beobachtete ich keine sicheren Effekte. Die angewendeten Konzentrationen lagen zwischen  $1 : 10^{-3}$  und  $1 : 10^{-13}$ .

In meinen Versuchen wurden während einer Stunde in der Regel 2,5 mg Histamin vernebelt, häufig bedeutend weniger. Nur ein Teil dieser Menge kann vom Versuchstier aufgenommen werden sein. Ferner ist zu bemerken, dass die Veränderungen der Geweboxydationen stets von dem Schweregrad und der Dauer des Asthmas abhingen, und nicht von der Histamin-Konzentration, die nötig war, um das Asthma auszulösen. Unter Beachtung dieser Befunde und mit Rücksicht u. a. auf die quantitativen Gesichtspunkte, dürfte man daher berechtigt sein, anzunehmen, dass die im experimentellen Asthma gefundene Herabsetzung der Geweboxydationen nicht einfach eine Folge der Histamin-Einwirkung ist. Man hat vielmehr alle Veranlassung dazu, die Veränderungen der Gewebsatmung für eine Folge der Stenoseatmung selbst zu halten. Diese Verhältnisse werden ebenfalls in Teil 2 dieses Kapitels näher behandelt werden.

## 2. Die Geweboxydationen bei Herabsetzung des Sauerstoff-Gehaltes und Erhöhung des Kohlensäure-Gehaltes in der Atmungsluft.

In Kapitel IV und V wurde gezeigt, dass die Sauerstoffaufnahme bei schwererem Asthma bedeutend sinkt. In Kapitel V wurde ferner nachgewiesen, dass die Sauerstoffsättigung des arteriellen Blutes auch bei einem leichteren Asthma abnimmt. Dies konnte unter den fraglichen Bedingungen nur durch eine Erschwerung des Gasaustausches in den Lungen erklärt werden. Die Erschwerung des Gasaustausches in den Lungen dürfte ihrerseits zum Teil dadurch erklärlich sein, dass im schweren Asthma die Lungenventilation pro Minute abnimmt (Kapitel V). Infolgedessen sinkt die alveolare Sauerstoffspannung und die alveolare Kohlensäurespannung steigt an. Auch bei leichterem Asthma dürften wir mit einer Verschiebung der Zusammensetzung

Curve 2 in the same figure is from a point in the moderately absorbing cytoplasm between the nuclear membrane and the periphery of the cell. At 2600 Å there is an absorption maximum and at 2800 a protein band. The tyrosin maximum lies at 2850 Å. The cytoplasm thus contains nucleotides as well as proteins of mainly basic character, in moderate concentrations. The extinction coefficients on an average are higher than the corresponding values in type I, but do not reach such high values as in type II.

**Feulgen reaction.** The different types of Purkinje's cells react to this test in the same way. Ribodesose particles are found only in the nucleus and show there the same characteristic distribution as in the analyzed ganglion cells. The largest groups lie close to the nucleolus in the form of minute rods. See Fig. 74. The nucleolus gives an entirely negative result, indicating that the nucleotides, amounting to about 2 per cent, must be of ribose type. The scanty amounts of Feulgen-positive particles in the remaining nuclear substance lie mainly in the vicinity of the nuclear membrane, as is shown also by Figure 74.

On a visual estimate, the content of Feulgen-positive particles in the different types of Purkinje's cells is quite similar. Though the Feulgen reaction does not admit of a quantitative determination of the amount of ribodesose nucleotides, it does enable us to estimate the number of particles containing ribodesose nucleotides in histological sections. In some cases, therefore, comparison with the results of the absorption measurements can give us some information as to the ratio between the ribose and the ribodesose nucleotides in a part of a cell. The nuclear substance, apart from the nucleolus, in type I contained nucleotides with an average concentration of about 2 per cent, whereas in the cells of type I the corresponding concentration was below 0.1 per cent. The Feulgen reaction showed no difference in the amount of Feulgen-positive particles in the two types of cell. This signifies that in type I *high concentrations of ribose nucleotides are contained not only in the nucleolus, but also in the remainder of the nuclear substance.*

The cytoplasm in the different types of Purkinje's cells gave an entirely negative response to the Feulgen reaction. The large aggregations of nucleotides close to the nucleus in type II are thus likewise of ribose type. They completely correspond, both in composition and location, with the nuclear-membrane nuc-

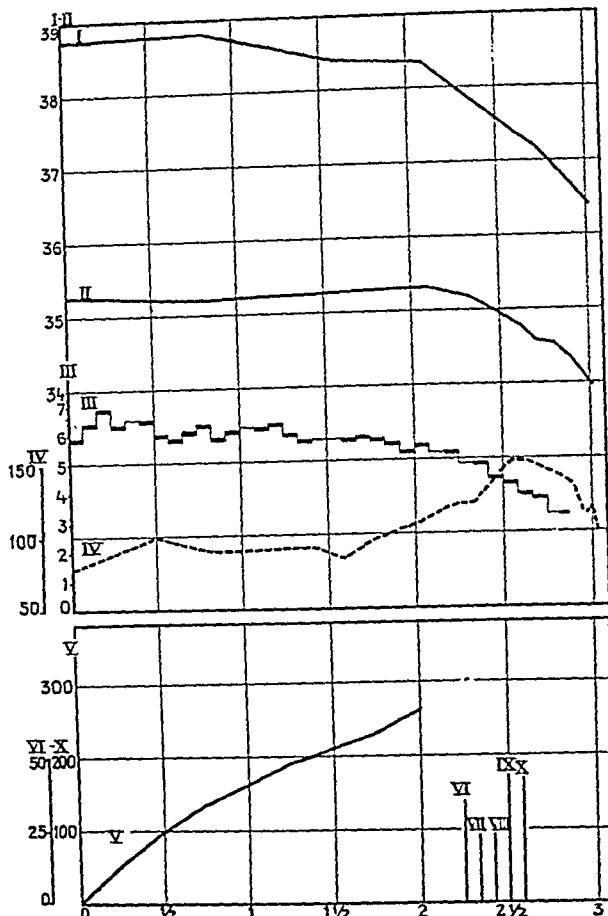


Abb. 20. Versuch vom 26. 2. 1943.

*Respirations-Versuch und Gewebsatmung bei Sauerstoffmangel.*

Allgemeinzustand des Versuchstieres: Ruhig. Während des Versuches tiefe, kräftige Atemzüge. Am Ende des Versuches sah das Tier matt aus.  
Zusammensetzung des Gasgemisches im System:

Zeit nach Beginn des Versuches	Kohlensäure- Gehalt	Sauerstoff- Gehalt
2 1/2 Std.	0,42 %	5,53 %
3 »	0,32 %	2,71 %

- I. Rektaltemperatur in Grad Celsius.
- II. Hauttemperatur in Grad Celsius.
- III. Sauerstoffaufnahme in cc pro Minute (reduziert).
- IV. Atemfrequenz pro Minute.

smaller. The area of the largest of the central sections comprising the nucleolus in the measured cells of that type averaged  $800 \mu$ . The real absorption at  $2600 \text{ \AA}$  for type I averaged  $\epsilon = 0.1$  and for type II  $\epsilon = 0.3$ . If this difference were due to shrinkage, it should amount to  $\left(\frac{0.1}{0.3}\right)^{\frac{1}{2}}$ , that is, 48 per cent.

However, the difference between the measured cells of types I and II did not exceed 25 per cent, whence the marked disparity in the nucleotide content cannot be due to a shrinkage caused by the treatment.

If we consider the previously described course of the irritation conditions in the endocellular protein-forming system, corresponding to different phases in the production of cytoplasmic proteins, the above reported cytochemical observations of the Purkinje's cells present the picture of a probable physiological process, in which the described types of cell correspond to different stages, and which leads to the building-up in the cell of nucleotides and proteins.

In the Purkinje's cells with a low content of nucleotides and proteins (type I) there are signs of an intense irritation of the protein-forming system in the form of a large nucleolus rich in nucleotides and of nuclear-membrane nucleotides in large concentrations. In the next phase, analogously with the results described in Chapters IV and V, this intense activity has resulted in charging both the nucleus and the cytoplasm with ribose nucleotides and basis proteins (the intermediate forms referred to above). In this way the picture of the Purkinje cell of type II, with ribose nucleotides and basic proteins in very large concentrations throughout the cell, has apparently been produced.



brane, during which ribose nucleotides are built up and, in connection therewith, also cytoplasmic proteins. Certain details in this mechanism were studied in the ganglion cells of fishes.

*Embryonic development.* During the embryonic development of the nerve cell two stages can be distinguished. During the first stage the embryonic nerve cells increase in number by mitosis. Then follows a period of growth without division during which the nerve cell, in regard to the development of the nucleolar and nuclear membrane apparatus, does not deviate from other somatic cells. To this stage the unipolar neuroblast belongs. Its nucleus contains large amounts of ribodesose nucleotides. The cytoplasm is but little developed and contains ribose nucleotides and basic proteins in moderate concentrations. To this stage belongs also the next step in the development on the nerve cell, the multipolar neuroblast, in which the amount of cytoplasmic proteins has moderately increased. In this type of cell one can observe a moderate increase of the total amount of protein in the cytoplasm, which, as compared with a unipolar neuroblast, contains, relatively to the protein, comparatively small amounts of nucleotides.

In both these embryonal stages a nucleolus proper is lacking. In the nucleus, on the other hand, there is a chromocentre-like area containing ribodesose nucleotides in large amounts. Thus far the development of the nerve cell completely corresponds with that of other somatic cells.

In the further course of its development, the nerve cell passes through a second period of growth, during which it increases in size and distinctly diverges from other somatic cells. During this period a large nucleolus rich in ribose nucleotides and an extensive apparatus of cytoplasmic nucleotides are developed. *In connection with the development of the nucleolus,* the author has been able to show a chromocentre area in the nucleus, analogous in composition to that of certain invertebrates, and containing particles rich in ribodesose nucleotides embedded in a ground-substance of proteins rich in hexone bases in high concentrations and of ribose nucleotides. In the adult nerve cell this chromocentre remains, lying close to the nucleolus. During this second period of growth the nucleolar apparatus passes through a very rapid development. Detailed analysis shows that the nucleolar and nuclear-membrane system is intensely active. The nucleolus is large and rich in ribose nucleotides, the nuclei are excentrically situated in the cell, the

Atemfrequenz steigt anfangs und beginnt gegen Ende des Versuches zu sinken (siehe Abb. 21). Wenn die Atemfrequenz auf 80 bis 100 pro Min. abgenommen hatte, wurden die Tiere in der Regel getötet. Die Zeit, die die Tiere unter der veränderten Gaszusammensetzung standen, betrug meist etwa  $1\frac{1}{2}$  bis 3 Stunden. Gegen Ende der Versuche sank die Haut- und Rektaltemperatur der Tiere. Dies geht aus dem in Abb. 21 wiedergegebenen Versuch hervor. Die Versuchstiere sehen zu dieser Zeit matt und elend aus und schnappen mit jedem Atemzug nach Luft. Es kam manchmal vor, dass die Tiere bei einer Atemfrequenz von 140 pro Min. plötzlich starben. Seziert man die Tiere unmittelbar, nachdem sie zusammengebrochen waren, so findet man meist, dass das Herz stark kontrahiert ist, ebenso wie man es sieht, wenn die Tiere in einem Histamin-Asthma, das längere Zeit anhielt, starben.

Nach der Einwirkung von Sauerstoffmangel und nach der gleichzeitigen Einwirkung von Sauerstoffmangel und Kohlensäure-Überschuss waren die verschiedenen Organe bei der Sektion stark zyanotisch, sie zeigten aber im übrigen keine makroskopischen Veränderungen.

Die erste orientierende Versuchsserie, die über das Verhalten der Geweboxydationen unter den fraglichen Bedingungen durchgeführt wurde, ist in Abb. 22 wiedergegeben.

In dieser Serie wurden ein Normalversuch (14. 1.), zwei Versuche, in denen das Versuchstier allein dem Sauerstoffmangel ausgesetzt wurde (9. 1. und 16. 1.), ein Versuch, in dem das Tier der Einwirkung eines Kohlensäure-Überschusses unterworfen wurde (12. 1.), und schliesslich zwei Versuche, in denen die Tiere unter der gleichzeitigen Einwirkung des Sauerstoffmangels und des Kohlensäure-Überschusses standen (10. 1. und 13. 1.), angestellt. Bei der Analyse der in Abb. 22 wiedergegebenen Versuchsserie ergibt sich folgendes:

**Leber:** Abb. 22 Nr. 1 ergibt sich, dass die Gewebsatmung der Leber in den beiden Versuchen, in denen das Tier gleichzeitig der Einwirkung des Sauerstoffmangels und des Kohlensäure-Überschusses ausgesetzt war, deutlich vermindert war. Dagegen zeigten sich keine sicheren Veränderungen der Geweboxydationen in der Leber unter den übrigen Versuchsbedingungen. Es

in structure. The nucleolus in these cells contains ribose nucleotides in large concentrations and at the periphery is surrounded by a number of particles rich in ribodesose nucleotides. Close to the nucleolus lies the above-described chromocentre. The remainder of the nuclear substance contains proteins rich in hexone bases, in the anterior horn cells in relatively large, in the spinal ganglion cells in rather small, concentrations. In some of the smaller spinal cells the nuclear substance also contains ribose nucleotides. The cytoplasm in the anterior horn cells contains large amounts of ribose nucleotides and proteins rich in hexone bases in very large concentrations. In the cytoplasm of the spinal ganglion cells these substances occur in much smaller concentrations.

*Motor irritation.* During intense muscular work a disappearance of the nucleotides and proteins in the cell could be observed in the root-cells concerned. From this it is inferred that increased activity of the nerve cell is accompanied by extensive process of protein and nucleotide metabolism. As a symptom of the irritation of the protein-producing system, the nucleolus is remarkably large and contains more basic groups than the nucleolus in cells from animals "at rest", whilst small quantities of protein, with a relatively large amount of basic groups, are found in the nuclear substance. This shows that, though the cell mechanism is adjusted for recuperative processes, they are unable, owing to the greater motor activity, to compensate the loss.

*Sensory irritation.* After faradic irritation of spinal ganglion cells, a marked increase in the cell's content of nucleotides and basic proteins, amounting to 2—3 times that of the non-irritated cell, as well as signs of an intense irritation of the nucleolar apparatus could at first be observed. The most probable explanation seems to be that during the protracted irritation the protein substances in the cell are steadily consumed, that the loss is met by the formation of new protein, but that this recuperative activity, owing to the drastic effects of the lengthy faradic irritation, does not suffice to compensate the breaking-down process: the cell is eventually drained of proteins. The experiments with faradic irritation thus show that it is actually possible to set the nucleolar apparatus in operation by artificial irritation, and the changes show the same general character as in prolonged motor irritation, namely a disappearance of the proteins in the cytoplasm.

*Niere:* Abb. 22 Nr. 2. Unter allen Versuchsbedingungen sind die Geweboxydationen normal und von gleicher Grösse, wie in den in Abb. 18 und 19 wiedergegebenen Versuchen. Ebenso wie die Gewebsöxydationen der Niere vom Histamin-Asthma nicht beeinflusst werden, scheinen auch unter den hier angewendeten Versuchsbedingungen die oxydativen Prozesse im Nierengewebe nicht verändert zu werden.

Die eben besprochene orientierende Versuchsserie schien darauf hinzuweisen, dass mindestens in bestimmten Organen die Geweboxydationen herabgesetzt werden, wenn die Versuchstiere vor ihrem Tode einer Veränderung in der Zusammensetzung der Einatmungsluft ausgesetzt wurden. Die Versuche der betreffenden Serie sprechen auch dafür, dass man in manchen Organen (Leber und Oberschenkelmuskulatur) bei der gleichzeitigen Einwirkung von Sauerstoffmangel und Kohlensäure-Überschuss leichter Veränderungen in den Geweben hervorrufen kann, die sich in einer Herabsetzung der oxydativen Prozesse manifestieren.

Da wir im experimentellen Asthma, wie weiter oben dargestellt, mit einer Herabsetzung der Sauerstoffspannung und Erhöhung der Kohlensäurespannung im Organismus rechnen müssen, untersuchte ich an einer grösseren Anzahl von Versuchstieren die Geweboxydationen, nachdem die Tiere der Einwirkung der beiden genannten Faktoren unterworfen wurden. Dies geschah, um ein genügend grosses Versuchsmaterial zu erhalten, das sichere Schlüsse zu ziehen erlaubte. Dagegen wurde nur eine geringere Zahl von Versuchen angestellt, in denen die Einwirkung des Sauerstoffmangels und des Kohlensäureüberschusses, jeder allein für sich, untersucht wurde. Die Ergebnisse der ausgeführten Versuche sind in Abb. 23 und 24 zusammengestellt. In diesen Versuchen wurden vor allem folgende Gewebe untersucht: Leber, Oberschenkelmuskulatur, Zwerchfell, Herzmuskel und Niere. Wie aus Abb. 23 hervorgeht, ist bei gleichzeitiger Einwirkung von Sauerstoffmangel und Kohlensäure-Überschuss die Herabsetzung des Sauerstoffverbrauches im Warburg-Versuch für die Leber, die Oberschenkelmuskulatur, das Zwerchfell und den Herzmuskel statistisch sichergestellt. Dagegen sind die Geweboxydationen in der Niere nicht vermindert. Im allgemeinen

in view of their special cytology and size, are particularly suited for investigation.

In a cross section through a part of the cerebellum, for the purpose of ultraviolet microscopy, the author observed in the same convolution Purkinje cells showing throughout (1) a *low* content of nucleotides and proteins and (2) a very *high* content of those substances in nucleus and cytoplasm. Between them there were cells holding an intermediate position in regard to composition, thus forming a transition between the two main types.

From Purkinje cells with a *low* content of nucleotides and proteins and — as a token of the irritation of the nucleolar apparatus —, well-developed nuclear-membrane nucleotides, one can follow the successive charging of the cell with nucleotides and basic proteins until it is converted into the second main type of the Purkinje cells, with a *high* content of proteins and nucleotides both in the nucleus and in the cytoplasm. The cytochemical pictures of the Purkinje cells are in good correspondence with the results obtained in the above reported experiments with artificial irritation.

From the above observations it may be inferred, with some degree of probability, that the Purkinje cell pictures represent different functional conditions.

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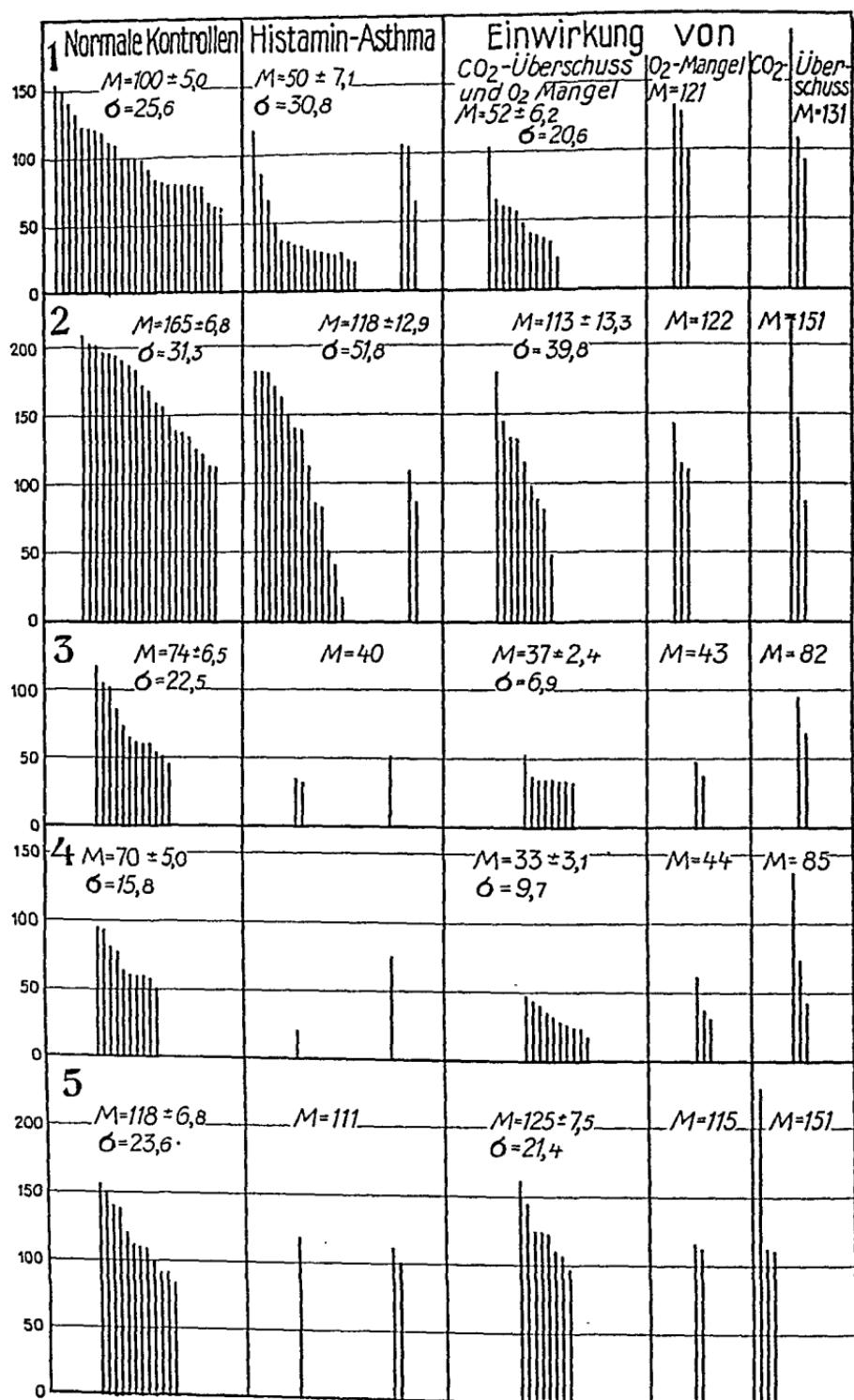


Abb. 23. (Siehe S. 132.)

ution, whilst the nucleolar system exhibited symptoms of intense irritation. This is a striking indication that the decrease in the proteins of the cell is closely associated with its function, and that reparative processes immediately set in, though, after intense activity or irritation, they are unable to make good the losses.

In spinal ganglion cells subjected to irritation with induced electric currents, new proteins are formed with surprising rapidity, but, if the irritation is prolonged for some length of time, the cytoplasm will eventually be drained of those substances. Here too the function of the intensely irritated cell appears to be correlated with an immense destruction of proteins and a recuperative process.

These observations, in short, show that intense protein-rebuilding processes, which may extend to the major part of the cell-body substance, are carried on in close connection with the function of the nerve cell.

These views are further borne out by studies of regenerative processes after nerve excision, and particularly by the fact that the rapid development of the organelles of the protein-forming system sets in precisely at the moment when the function of the cell is beginning to be restored.

Finally, in the study of a special case, namely Purkinje's cells taken from the cerebellum of rabbits and cats, it has been shown that under physiological conditions cytochemical cell pictures are found which entirely correspond with the observations made in regard to cells in relatively inactivity or with those which have been set into intense activity.

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I desire to acknowledge my great indebtedness to Docent TORBJÖRN CASPERSSON for the interest he has shown in these investigations and for his valuable advice and criticism.

My thanks are also due to the Rockefeller Foundation, Stiftelsen Therese och John Anderssons Minne and A. F. Regnells Fond, for financial support which has made it possible to me to carry out these investigations. The entire cost of translation has been kindly defrayed by the British Council.

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Abb. 23. *Übersichtsbild der mit der Warburg-Methode ausgeführten Versuche.* Jeder Stab representiert einen Versuch. Die Stäbe sind nur nach ihrer Länge geordnet. In der Rubrik Histamin-Asthma wurden die Versuche, in denen der Asthma-Anfall weniger als 5 Minuten andauerte, in einer besonderen Gruppe zusammengestellt. Diese Versuche wurden aber in die Berechnung der Mittelwerte und der mittleren Fehler mitaufgenommen.<sup>1</sup>

1. Leber.
2. Oberschenkelmuskulatur.
3. Zwerchfell.
4. Herzmuskel.
5. Niere.

Ordinate: Sauerstoffaufnahme in cmm während der ersten halben Stunde.

Abb. 24. *Übersichtsbild über die ausgeführten Methylenblau-Versuche.* Jeder Stab representiert einen Versuch. Die Stäbe sind der Grösse nach geordnet.

In jeder Rubrik gibt es drei Gruppen:

Gruppe I: 1000 γ Methyleneblau in 5 cc.			
Gruppe II: 1250 γ	»	»	5 cc.
Gruppe III: 1000 γ	»	»	1,3 cc.

1. Leber.
2. Oberschenkelmuskulatur.
3. Zwerchfell.
4. Herzmuskel.
5. Niere.

Ordinate: Entfärbungszeiten in Minuten.

den Lungen Veränderungen der Sauerstoff- und Kohlensäuretension des Blutes auf, die ihrerseits gemäss den hier dargestellten Versuchen im Stande sind, die Geweboxydationen zu senken.

Wie früher erwähnt, konnte BÜNGELER (1934) nach grossen Histamin-Dosen nur dann eine Herabsetzung der Geweboxyda-

<sup>1</sup> Bei der Einwirkung von Kohlensäure enthält die Abb. einen durchgehend auffallend hohen Wert. Dieser stammt von einem Versuch, der nach Abschluss der übrigen Versuchsreihen mit einem albinotischen Tier, das von einem anderen Tierhändler geliefert wurde, ausgeführt wurde. Zu den früheren Versuchen wurden stets gefärbte Tiere verwendet. Es wäre möglicherweise richtiger, diesen Wert auszuschliessen, zumal da keine Kontrollversuche mit gleichartigen Tieren ausgeführt werden konnten.

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*websoxydationen wesentlich ist, aber mindestens in manchen Fällen muss der Sauerstoffmangel durch einen gleichzeitigen Kohlensäure-Überschuss unterstützt werden.* Der Entstehungsmechanismus dieser Veränderungen soll in späteren Untersuchungen eingehend behandelt werden.

### 3. Die anaerobe Glykolyse.

In einigen Versuchsserien wurden Bestimmungen der anaeroben Glykolyse durchgeführt, vor allem in der Leber, da diese das Organ ist, in dem mit grösster Regelmässigkeit die stärkste Herabsetzung der Gewebeoxydationen feststellbar war. Diese Versuche wurden nur angestellt, um eine Vorstellung zu gewinnen, ob die Veränderungen der *in vitro* untersuchten anaeroben Glykolyse mehr oder weniger ausgesprochen waren, als die nach WARBURG und THUNBERG festgestellten Veränderungen der Gewebeoxydationen. Tabelle 3 gibt die erhaltenen Werte von einigen Versuchsserien wieder. Die angegebenen Werte stellen die Kohlensäure-Produktion von 200 mg Gewebsbrei in der ersten Stunde dar (siehe auch Tabelle 7).

*Tabelle 3.*

#### *Glykolyse-Versuche.*

Datum	Art des Versuches	emm	CO <sub>2</sub>
26.1. 1943.	Normal	31	26
28.1. 1943.	Histaminasthma	47	48
29.1. 1943.	Histaminasthma	41	35
1.2. 1943.	Normal	33	33
2.2. 1943.	O <sub>2</sub> -Mangel + CO <sub>2</sub> -Überschuss	34	31
22.2. 1943.	O <sub>2</sub> -Mangel + CO <sub>2</sub> -Überschuss	24	19
23.2. 1943.	Normal	32	
26.2. 1943.	O <sub>2</sub> -Mangel	31	

Vergleicht man die gefundenen Werte für die Glykolyse mit den entsprechenden Veränderungen in den Gewebeoxydationen, so gewinnt man die Auffassung, dass die Veränderungen in der anaeroben Glykolyse, wenn man solche überhaupt feststellen

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Tabelle 4.

Datum	Art des Versuches	Milchsäure in mg%	Brenztraubensäure in mg%
8. 2. 43.	Normal	25	3,1
9. 2. 43.	Normal	25	2,5
10. 2. 43.	Leichtes anaphylaktisches Asthma	28	2,5
22. 2. 43.	Sauerstoffmangel und Kohlensäure-Überschuss		2,7
23. 2. 43.	Normal		3,2
26. 2. 43.	Sauerstoffmangel	154	5,4
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der Glykogen-Gehalt der Leber im anaphylaktischen Schock erheblich ab, wenn dieser Zustand länger als 10 Minuten andauert. Dies dürfte wahrscheinlich so gedeutet werden, dass eine Glykogenmobilisierung aus der Leber zu Gunsten anderer Organe stattfindet.

In Übereinstimmung mit den Ergebnissen arbeitsphysiologischer Untersuchungen sollte die Glykolyse im Beginn eines schwereren Asthmas vollkommen dem Ablauf der Glykolyse bei gewöhnlicher schwerer Muskelarbeit in ihrer ersten, partiell anaeroben Phase entsprechen, dem Zustand, den man in den ersten Minuten nach Beginn der Arbeit beobachten kann, bevor sich die Lungen und der Kreislauf den erhöhten Ansprüchen angepasst haben (siehe BANG, 1935). Im weiteren Verlauf eines experimentellen Asthmas oder unter der langdauernden Einwirkung eines Sauerstoffmangels und eines Kohlensäure-Überschusses wäre es nicht unmöglich, dass die Glykose *in vivo* gehemmt sein könnte. Am anoxybiotisch arbeitenden Froscherzen zeigte nämlich ABDON (1942), dass sich die Cophosphorylase-Aktivität *in vivo* rasch verschlechtert.

Es wäre möglich, durch Belastungsversuche mit Milchsäure, bezw. mit Glykose eine nähere Aufklärung über das Verhalten der Glykolyse *in vivo* beim experimentellen Asthma zu erhalten. Derartige Versuche mussten aber für eine spätere Arbeit zurückgestellt werden.

*ACTA PHYSIOLOGICA SCANDINAVICA*  
VOL. 6 SUPPLEMENTUM XVIII

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AUS DEM PHARMAKOLOGISCHEN INSTITUT DER UNIVERSITÄT LUND

GASWECHSEL UND GEWEBSATMUNG  
BEIM EXPERIMENTELLEN ASTHMA  
DES MEERSCHWEINCHENS

EIN BEITRAG ZUR PATHOPHYSIOLOGIE  
DER STENOSEATMUNG

von

**HELGE COLDAHL**

LUND

## KAP. VII.

### Die Einwirkung einiger Substanzen auf die erniedrigte Gewebsatmung.

Im vorhergehenden Kapitel wurde gezeigt, dass bei Meerschweinchen, die unter der Einwirkung einer Verminderung der Sauerstoffspannung und einer Erhöhung der Kohlensäurespannung im Blute standen, die Gewebesoxydationen *in vitro* herabgesetzt sind. Man darf vermuten, dass diese Senkung der oxydativen Prozesse auf einer Störung der enzymatischen Prozesse in den Zellen beruht. Wäre dies in der Tat der Fall, so wäre es von grossem Interesse, festzustellen, ob die betreffende Schädigung generell ist, oder ob sie sich auf bestimmte enzymatische Systeme beschränkt.

In diesem Kapitel wird über Versuchsergebnisse berichtet werden, aus denen hervorgeht: In den Geweben von Versuchstieren, die unter der Einwirkung einer erhöhten  $\text{CO}_2$ -Spannung und einer herabgesetzten  $\text{O}_2$ -Spannung des Blutes standen, erwies sich ein bestimmtes Enzymsystem, *in vitro* untersucht, als ungeschädigt, während ein anderes geschädigt sein dürfte. Denn bei letzterem konnte eine Schädigung eines für dieses Enzymsystem notwendigen Coenzyms nachgewiesen werden.

Im ersten Abschnitt dieses Kapitels wird der Effekt behandelt, den der Zusatz von Laktat und von Succinat auf die Herabsetzung der Gewebesoxydationen ausübt. Im zweiten Abschnitt wird die Wirkung des Zusatzes von Cozymase und Cocarboxylase besprochen. Die Effekte wurden in der Regel nur an der Leber untersucht. Eine geringere Anzahl von Versuchen wurde auch an anderen Geweben durchgeführt, aber hier soll nur über die an der Leber ausgeführten Versuche berichtet werden.

*Meiner Mutter*

Man nahm an, dass die Cytochrome die physiologischen Äquivalente des Methylenblaus wären. Die Verhältnisse dürften aber in dieser Beziehung noch nicht völlig geklärt sein; denn reduziertes Diaphorase-Flavoprotein reagiert nach STRAUB nicht mit Cytochrom C. Auch wird das Diaphorase-Flavoprotein nicht durch C<sub>4</sub>-Dicarbonsäurekatalyse reoxydiert (vergl. STRAUB, 1942).

*Die Einwirkung des Zusatzes von Succinat und Laktat auf die Geweboxydationen.*

Nach Zusatz von Succinat in einer Endkonzentration von m/20 zum Lebergewebe im Normal-Versuch findet man eine starke Erhöhung der Sauerstoffaufnahme beim Warburg-Versuch (Abb. 26 und 27). Setzt man die gleiche Menge von Succinat dem Lebergewebe von Tieren zu, die unter der Einwirkung eines Kohlensäure-Überschusses und Sauerstoffmangels standen, und deren Sauerstoffaufnahme infolgedessen herabgesetzt war, so steigt die Sauerstoffaufnahme so stark an, dass sie ebenso gross wird, wie die des normalen Lebergewebes nach Succinatzusatz (Abb. 25 und 27). Die prozentuale Erhöhung der Sauerstoffaufnahme nach dem Zusatz von Succinat ist demnach erheblich stärker, wenn die Sauerstoffaufnahme des Lebergewebes vor dem Zusatz herabgesetzt war, als wenn sie vorher normal war.

Der Zusatz von Succinat zu normalem Lebergewebe im Methylenblau-Versuch erhöht die Oxydations-Intensität ein wenig. Die Erhöhung scheint in der Regel stärker zu sein, wenn das Succinat zu Lebergewebe zugesetzt wird, dessen Entfärbungszeit nach der Einwirkung eines Kohlensäure-Überschusses und Sauerstoffmangels auf die betreffenden Versuchstiere verlängert ist. In dem in Abb. 25 wiedergegebenen Versuch betrug z. B. die Entfärbungszeit für die Leber 83 Minuten, und für die Leber + Succinat 37 Minuten, während in dem in Abb. 26 wiedergegebenen Normal-Versuch die Entfärbungszeit der Leber 27 Minuten war, und die der Leber + Succinat 22 Minuten.

In Abb. 28 sind einige Versuche mit schwächeren Succinat-Konzentrationen dargestellt. In ihnen ist der Effekt auf die Sauerstoffaufnahme erheblich geringer. Bei einer Endkonzentration von m/540 (II in Abb. 28) ist die Wirkung nur angedeutet,

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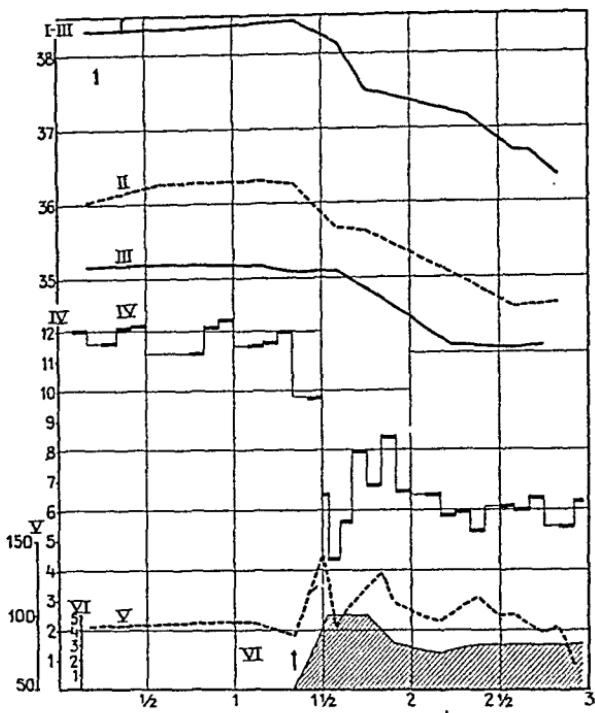


Abb. 25. Versuch vom 29. 6. 1942. Respirationsversuch und Gewebeoxydationen im Histamin-Asthma. Das Versuchstier atmete in atmosphärischer Luft. Asthma wurde durch 1 %ige Histamin-Lösung ausgelöst.  
Allgemeinzustand des Versuchstieres: Ruhig während des Versuches.

1. *Histamin-Asthma im geschlossenen System.*

- I. Rektaltemperatur in Grad Celsius.
- II und III. Hauttemperaturen in Grad Celsius.
- IV. Sauerstoffaufnahme in cc pro Minute (reduziert).
- V. Atemfrequenz pro Minute.
- VI. Schweregrad des Asthmas.

2. *Gewebeoxydationen in der Leber.*

- I. Sauerstoffaufnahme im Warburg-Apparat in cmm für Leber + m/22 Succinat.
- II. Sauerstoffaufnahme im Warburg-Apparat in cmm für Leber + m/27 Laktat + 1,5 mg Cozymase II.
- III. Sauerstoffaufnahme im Warburg-Apparat in cmm für Leber + m/27 Laktat + 0,5 mg Cozymase II.
- IV. Sauerstoffaufnahme im Warburg-Apparat in cmm für Leber + m/27 Laktat.

## V O R W O R T

Vorliegende Arbeit ist aus Untersuchungen hervorgegangen, die ich in den Jahren 1939 bis 1943 als Assistent am Pharmakologischen Institut der Kgl. Universität Lund ausgeführt habe. Ein Teil der Versuchsergebnisse wurde bereits in vorläufigen Mitteilungen veröffentlicht.

Dem Vorstand des Institutes, Herrn Professor Dr. GUNNAR AHLGREX, habe ich in erster Linie dafür zu danken, dass er mir bereitwilligst gute Arbeitsbedingungen geschaffen hat, sowie für sein stetes Interesse während der Durchführung der Arbeit.

Ferner danke ich Herrn Professor Dr. KARL MYRBÄCK für die Liebenswürdigkeit, die er mir während eines Arbeitsaufenthaltes in seinem Institut in Stockholm erwies. Herrn Professor Dr. C.-E. QUENSEL bin ich für seine Ratschläge bei der statistischen Bewertung meiner Versuchsergebnisse zu Dank verpflichtet.

Meinen Kollegen im Institut danke ich sehr für wiederholte Ratschläge und Hilfe. Für wesentliche Unterstützung bei der Konstruktion und Handhabung meiner Versuchsapparatur habe ich dem Instrumentenmacher des Institutes, Herrn PER LARSSON, zu danken.

Für unermüdliche technische Assistenz im Laboratorium danke ich meiner Schwester, Frau BRITTA JÄDERSTRÖM, und Frau INGRID ENGBERG. Bei der Durchrechnung der Versuchsergebnisse und ihrer graphischen Darstellung hat mir mein Bruder, Kapitän GUNNAR COLDAHL, in wertvoller Weise geholfen.

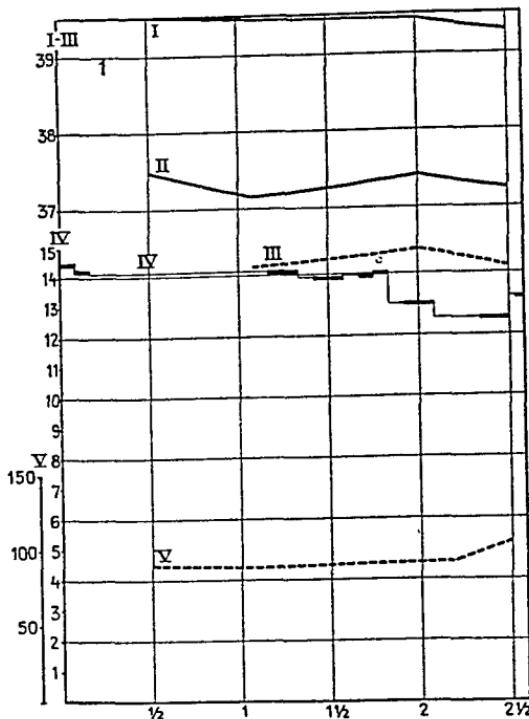


Abb. 26. Versuch vom 1. 7. 1942. Respirationsversuch und Gewebeoxydationen im Kontrollversuch zu Abb. 25. Bezeichnungen und Konzentrationen wie in Abb. 25, soweit nicht anders angegeben. Das Versuchstier atmete in atmosphärischer Luft.

Allgemeinzustand des Versuchstieres: Ruhig während des Versuches.

1. *Respirationsversuch.*

2. *Gewebeoxydationen der Leber.*

- II. Sauerstoffaufnahme im Warburg-Apparat in cmm für Leber + m/27 Laktat + 3 mg Cozymase II.
- III. Sauerstoffaufnahme im Warburg-Apparat in cmm für Leber + m/27 Laktat + 0,05 mg Cozymase II.

3. *Gewebeoxydationen der Oberschenkelmuskulatur.*

Bezeichnungen wie bei der Leber.

(Siehe auch Tabelle 7.)

Es ist interessant, dass man nach längerem Hunger (z. B. im Versuch vom 15. 12. 1942, in dem das Tier etwa 40 Std. gehungert hatte) eine Herabsetzung der Sauerstoffaufnahme der Gewebe im Warburg-Versuch finden kann. Der Ausfall des

## **Einleitung.**

Bei genügend starker Einengung der Luftwege entsteht eine Dyspnoe, die oft als Stenoseatmung bezeichnet wird. Je nach der Lokalisation des Hindernisses manifestiert sie sich etwas verschieden.

Experimentell kann man Stenoseatmung auf verschiedene Weise hervorrufen. Zwei Typen sind vor allem zu unterscheiden: 1.) Eine Stenoseatmung kann dadurch ausgelöst werden, dass man die Versuchsperson oder das Versuchstier durch ein zu enges Mundstück resp. durch eine eingeengte Trachealkanüle atmen lässt. Diese Form wird gewöhnlich »mechanische Stenoseatmung« genannt. 2.) Man kann Stenoseatmung dadurch hervorrufen, dass auf die eine oder andere Weise Stoffe in den Körper eingeführt werden, die das Bronchiallumen verengern. Zu dieser Gruppe gehört die Stenoseatmung im anaphylaktischen Schock und beim experimentellen Asthma. Bei letzterem wird die Atmungsveränderung durch Inhalation ausgelöst.

Beim Menschen kommt Stenoseatmung bei einigen Erkrankungen vor. Sie beruht auf verschiedenartigen Einengungen an verschiedenen Teilen der Atmungswege. Zu diesen Erkrankungen gehören Asthma bronchiale, kapilläre Bronchitis, Krupp, Pseudokrupp und Tumoren an den Atmungswegen selbst oder in ihrer Nachbarschaft. Hierbei gleicht die Stenoseatmung bei einem Teil der Fälle am meisten der mechanischen Stenoseatmung (z. B. bei Tumoren, die die Trachea einengen), bei anderen Fällen mehr dem Typus der Atmungsveränderung, der durch Pharmaka oder im anaphylaktischen Schock ausgelöst wird (z. B. bei gewissen Formen des Asthma bronchiale).

Es gibt experimentelle und klinische Untersuchungen, die zeigen, dass es bei der Stenoseatmung zu Veränderungen der

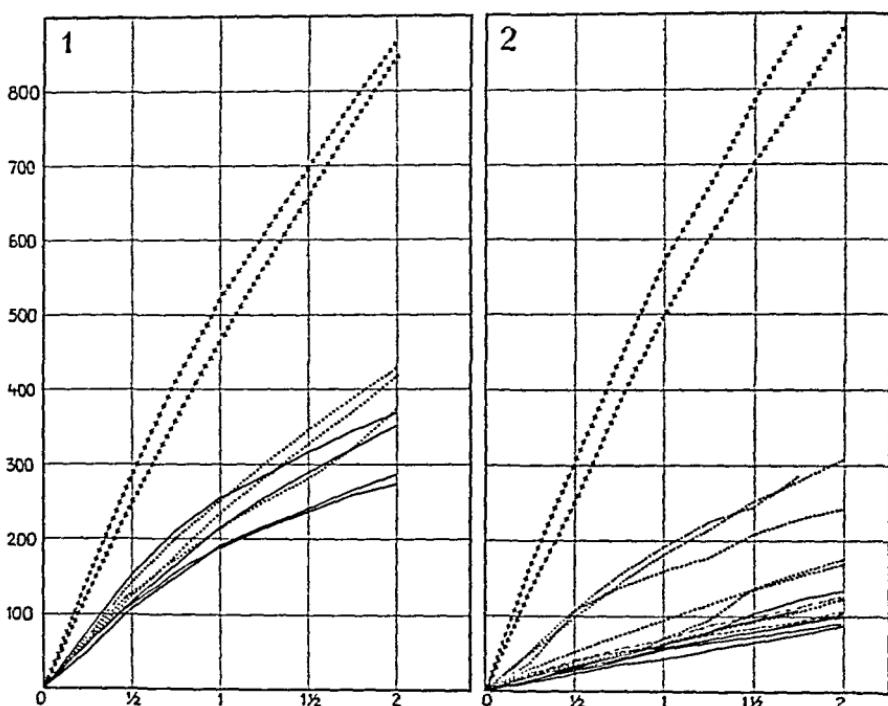


Abb. 27. Versuche über das Verhalten der Gewebeoxydationen bei Zusatz von grossen Mengen Succinat und Laktat. Endkonzentrationen waren für Laktat m/27 und für Succinat m/20.

1. Normalversuche.

- Kein Zusatz.
- ..... Zusatz von Laktat.
- +++ Zusatz von Succinat.

2. a. Asthma-Versuche.

- Kein Zusatz.
- ..... Zusatz von Laktat.
- +++ Zusatz von Succinat.

b. Sauerstoffmangel und Kohlensäure-Überschuss.

- — — Kein Zusatz.
- · — Zusatz von Laktat.

(Siehe auch Tabelle 7.)

hinweisen, dass unter den fraglichen Bedingungen die Succinat-Oxydation *in vivo* erschwert ist. Die einfachste Erklärung für diese Erscheinung dürfte die sein, die von FORSSMAN gegeben wurde, nämlich dass Sauerstoffmangel die Ursache für das Fehlen der Succinat-Oxydation *in vivo* wäre. In diesem Falle

Untersuchungen behandelt, die über den oxydativen Gewebsstoffwechsel bei diesen Zuständen vorliegen, da manche Autoren Veränderungen der Oxydationen in den Geweben für die primäre Ursache der Veränderungen des Gasaustausches halten.

## KAP. I.

### Literaturübersicht und Fragestellung.

#### 1. Mechanische Stenoseatmung.

KÖHLER (1877) untersuchte an Kaninchen, Katzen und Hunden die Veränderungen der Lungenventilation und Atemfrequenz, die bei mechanisch hervorgerufener Stenoseatmung auftreten. Bei Kaninchen wurde ein Bleidraht um die Trachea gelegt. Die durch die Zuklemmung des Bleidrahtes entstehende Dyspnoe wurde bis zu 14 Tagen nach Anlegung der Stenose untersucht. Bei Katzen und Hunden wurden nur akute Versuche ausgeführt, und die Stenoseatmung durch eine Einengung der Trachealkanüle bewirkt. Die Trachealkanüle wurde in allen Versuchen mit einem Apparat zur Bestimmung der Lungenventilation pro Minute verbunden. Mittels eines Czermak'schen Doppelhebels, der auf dem Sternum der Versuchstiere angebracht wurde, wurde der Luftstrom im Apparat so geleitet, dass die Tiere in der einen Richtung ein-, in der anderen ausatmeten. Durch diese Anordnung wurde das Hindernis, das ein Atmungsventil darstellen kann, vermieden. Man kann aber gegen dieses Vorgehen einwenden, dass der Doppelhebel einen gewissen Widerstand gegen die Thoraxexkursionen ausüben kann, und dass ausserdem z. B. eine extreme Ausdehnung des Thorax nicht immer mit der Einatmung von Luft einhergeht. Diese Verhältnisse dürften aber die Versuchsresultate kaum wesentlich beeinflusst haben. Die Versuchstiere wurden vor Beginn des Versuches auf den Operationstisch geschnallt. Über die angewandte Narkose wird nichts mitgeteilt. KÖHLER fand bei allen untersuchten Tierarten während der Stenoseatmung eine Herabsetzung der Atemfrequenz und eine Erhöhung des Atemvo-

lumens. Bei den Kaninchen ist die Frequenzabnahme so stark, dass die Lungenventilation pro Minute abnimmt. Bei den Katzen und Hunden nimmt die Atemfrequenz während der Stenoseatmung bedeutend weniger ab, so dass bei diesen Tierarten die Lungenventilation pro Minute gleichzeitig ansteigt.

MORAWITZ und SIEBECK (1909) untersuchten bei drei Versuchspersonen den Effekt der Stenoseatmung. Diese wurde durch Zuklemmen einer Schlauchkupplung hervorgerufen, die an dem Mundstück befestigt war, durch das die Versuchspersonen atmeten. Bei zwei dieser Versuchspersonen wurde die Lungenventilation pro Minute grösser, bei der Dritten kleiner. Die Atemfrequenz nahm bei allen drei Fällen während der Stenoseatmung ab.

MOORE und BINGER (1927) untersuchten an Hunden in Diätylbarbitursäurenarkose die Wirkung einer mechanischen Stenoseatmung, die durch Einführung eines Widerstandes für die Atmungsluft teils im Inspirations- teils im Exspirations-Schlauch hervorgerufen wurde. Die Versuchstiere wurden in einer Vorperiode, während der Stenoseatmung, die 1 bis 2 Stunden anhielt, und in einer Nachperiode nach ihrem Aufhören beobachtet. Wurde die Inspiration behindert, so stieg die Atemfrequenz an, während das Atemvolumen und die Lungenventilation pro Minute kleiner wurden. Die arterielle Sauerstoffsättigung sank während der Stenoseatmung auf etwa die Hälfte, auch bei Einatmung eines Gasgemisches mit 95 % Sauerstoff. Die Kohlensäurespannung des arteriellen Blutes stieg an, und das  $p_H$  sank in manchen Fällen bis zu 0,2 Einheiten. Nach Entfernung des Atmungshindernisses blieb die Atemfrequenz noch längere Zeit erhöht, während gleichzeitig das Atemvolumen und die Lungenventilation pro Minute zunahmen. Letztere erreichte in manchen Versuchen recht hohe Werte. Wurde dagegen die Expiration behindert, so nahm die Atemfrequenz ab. Die Lungenventilation pro Minute wurde auch in diesem Fall kleiner. In einem Teil der Versuche sank auch unter diesen Bedingungen die arterielle Sauerstoffsättigung und das  $p_H$  des arteriellen Blutes, während die Kohlensäurespannung anstieg. Diese Veränderungen waren aber nicht so regelmässig zu beobachten, wie bei Behinderung der Inspiration. Nach Entfernung des Atemhinder-

nisses wurden alle untersuchten Größen allmählich wieder normal. Bei der Obduktion wurde nach Behinderung der Inspiration Blutüberfüllung der Lungen und Lungenödem gefunden. Nach Behinderung der Expiration konnten dagegen keine charakteristischen Veränderungen der Lungen beobachtet werden.

ANTHONY (1928) untersuchte mittels des Knippingapparates die Lungenvентilation pro Minute, das Atemvolumen und die Respirationsfrequenz bei verschiedenen Graden von Stenose. Diese wurden dadurch bewirkt, dass ein Hahn, der zwischen der Versuchsperson und dem Apparat eingesetzt war, in verschiedene Stellungen gedreht wurde. Während der Stenoseatmung wurde das Atemvolumen bei 7 von insgesamt 8 Versuchspersonen erheblich verkleinert. Die Respirationsfrequenz verminderte sich meist etwas, wenn auch nur unwesentlich. Die Lungenventilation pro Minute nahm deutlich ab. Ferner wurde während der Stenoseatmung die Zeitdauer für jede Ein- und Ausatmung etwas verlängert, gleich stark für jede der Atemphasen. Gleichzeitig wurde die Normalkapazität der Lungen nach Rohrer (d. h. der Luftgehalt der Lungen in Expirationsstellung) erhöht.

Mitteils einer pneumotachographischen Methode zeigte SCHNEYER (1930), dass es beim Menschen unter der Stenoseatmung mit zunehmender Behinderung der Atmung zwei verschiedene Reaktionstypen bezüglich der Atemveränderungen gibt. In der Regel wird das Atemvolumen erhöht, die Atemfrequenz herabgesetzt. Bei einem Teil der Versuchspersonen nimmt dagegen die Atemfrequenz zu, während das Atemvolumen verkleinert wird. In beiden Fällen wird die Lungenventilation pro Minute etwas herabgesetzt.

LIPPELT (1932) konnte ANTHONY's Versuchsresultate mit der gleichen Methode an mittelschweren und schweren Stenosen bestätigen. Bei geringgradigen Atemstenosen fand er dagegen eine leichte Erhöhung der Lungenventilation pro Minute.

LUDWIG (1939) untersuchte mittels des Knipping'schen Apparates die Einwirkung von Gasmasken auf die Lungenventilation pro Minute, die Atemfrequenz, den Sauerstoffverbrauch u. a. Seine Beobachtungen umfassen jeweils 10-Minutenperioden.

Der Verf. untersuchte dabei teils allein den Effekt der Vergrösserung des schädlichen Raumes (um 250 ccm), teils den der stenosierenden Einwirkung des Filters allein, und schliesslich wurde die Beeinflussung der Atmung durch die komplette Gasmaske studiert. Letztere stellt die Kombination der Wirkungen des schädlichen Raumes und der Atembehinderung durch Stenosierung dar. Unter allen drei Versuchsbedingungen wurde eine Erhöhung des Atemvolumens pro Minute von etwa 7 auf 10 bis 11 Liter gefunden. Der Sauerstoffverbrauch pro Minute nahm dagegen ab, bei Anwendung der ganzen Gasmaske von 3,23 auf 2,61 Liter für eine 10-Minutenperiode. Bei der Stenoseatmung wurde der respiratorische Quotient leicht erhöht. Ludwig behauptet, dass in der Literatur keine Angaben über Herabsetzung des Stoffwechsels bei Behinderung der Einatmung vorliegen. Er nimmt an, dass es sich hier um eine reflektorische Einwirkung auf die Stoffwechsel-Zentra handele, die eine »Sparmassnahme« darstelle.

Mittels der Douglas'schen Methode untersuchte MATTHES (1940) die Lungenventilation pro Minute und den Sauerstoffverbrauch von Versuchspersonen, die auf einer Tretbahn arbeiteten, und deren Atmung gleichzeitig durch Verengerung des Inspirations- oder Expirationsschlauches in verschiedenen starkem Grade behindert wurde. Die Versuchspersonen ruhten zunächst 20 Minuten, dann gingen sie 3 Minuten auf der Tretbahn und atmeten danach 3 Minuten in einen Douglassack. Der Verf. fand, dass die Lungenventilation pro Minute und der Sauerstoffverbrauch grösser als normal wurde, wenn der Atmungswiderstand unter 100 mm Wasser war. Wurde der Widerstand über diese Grenze hinaus erhöht, so nahmen Lungenventilation wie Sauerstoffverbrauch ab. Der respiratorische Quotient stieg bei der Stenoseatmung etwas an. Im Gegensatz zu MOORE und BINGER fand MATTHES, dass bei schweren Stenosen die Atemfrequenz unter Behinderung der Inspiration stärker abnahm, als bei Erschwerung des Expiriums. Die Erhöhung des Sauerstoffverbrauchs bei leichteren Graden von Stenoseatmung beruht nach der Ansicht des Verf. darauf, dass durch die Atmungsbehinderung die Atemarbeit erhöht wird. Bei schwereren Graden von Stenosierung dagegen soll die Ventilation nicht dem Bedarf

entsprechen, und infolgedessen der Sauerstoffverbrauch sinken. Hierbei sollte die Muskelarbeit zum Teil anaerob unter Vermehrung der Milchsäurebildung erfolgen, wofür aber vom Verf. keine experimentellen Belege gegeben werden. Die Versuchspersonen sind übrigens auch bei Erhöhung des Atemwiderstandes über 100 mm Wasser im Stande, ihre Ventilation zu erhöhen, wenn man sie hierzu energisch auffordert. Die Tatsache, dass die Versuchspersonen ihre Ventilation nicht spontan vergrössern, soll darauf beruhen, dass unter diesen Bedingungen ein zu schwacher Reiz auf das Atemzentrum ausgeübt wird. Eigentümlicher weise wird aber die Lungenventilation pro Minute und der Sauerstoffverbrauch wieder vergrössert, wenn die Versuchsperson eine langdauernde Muskelarbeit so lange fortsetzt, bis sie nicht mehr weiter kann, also bis zur Erschöpfung. MATTHES erklärt dies Phänomen so, dass die Sauerstoffschuld nicht beliebig erhöht werden kann. Hat letztere ein Maximum erreicht, so muss die Sauerstoffaufnahme wieder ansteigen. Bevor man diesen Schluss zieht, wäre es aber notwendig, durch Milchsäure-Bestimmungen die Grösse der Sauerstoffschuld zu untersuchen, was nicht geschehen ist. Im Gegensatz zu LUDWIG nimmt MATTHES an, dass die Herabsetzung der Sauerstoffaufnahme bei Stenoseatmung eine Folge der ungenügenden Lungenventilation ist. Als Beweis hierfür führt er an, dass die Leistungsfähigkeit bei Stenoseatmung herabgesetzt ist.

Fasst man die Versuchsergebnisse zusammen, die bezüglich des Gasaustausches an Menschen und an Versuchstieren während einer auf mechanische Weise ausgelösten Stenoseatmung gewonnen wurden, so ist zu sagen: Direkte Bestimmungen der Sauerstoffaufnahme während der Stenoseatmung wurden nur an Menschen ausgeführt. LUDWIG fand hierbei eine Herabsetzung der Sauerstoffaufnahme. Das Gleiche beobachtete MATTHES unter gewissen Umständen, nämlich bei schwererer Behinderung der Atmung, soweit diese nicht bis zur Erschöpfung fortgesetzt wurde. Bei geringgradiger Atembehinderung dagegen ist der Sauerstoffverbrauch nach MATTHES erhöht. Was die Lungenventilation pro Minute betrifft, stimmen die Angaben der verschiedenen Autoren nicht überein. LUDWIG fand eine Erhöhung, MOORE und BINGER, ANTHONY sowie SCHNEYER eine Herabsetzung

der Lungenvентilation pro Minute während der Stenoseatmung. KÖHLER, MORAWITZ und SIEBECK, LIPPELT sowie MATTHES beobachteten bei manchen Fällen eine Vergrösserung, bei anderen eine Verkleinerung des Atemminutenvolumens. Meist nahm diese Grösse bei leichten Graden von Atemstenose zu und verminderte sich bei schweren Stenosen.

## 2. Anaphylaktischer Schock.

LOENING (1911) zeigte an Meerschweinchen und Kaninchen in schwerem anaphylaktischem Schock, nach intraperitonealer Reinfektion, dass die Sauerstoffaufnahme erheblich herabgesetzt war, während der respiratorische Quotient meist anstieg. Die Körpertemperatur der Versuchstiere konnte im schweren Schock um mehrere Grade sinken. Einen kompensatorischen Anstieg der Sauerstoffaufnahme nach dem Abklingen der anaphylaktischen Reaktion lassen die Versuche von LOENING nicht erkennen. Bei leichter anaphylaktischer Reaktion an Kaninchen, die ungenügend sensibilisiert waren, stieg dagegen der Sauerstoffverbrauch an, und die Körpertemperatur zeigte eine leichte Erhöhung. LOENING wendete die Methode von Haldane an. Er bestimmte vor Eintritt, während des anaphylaktischen Schockes und nach seinem Abklingen jeweils in 2-Stunden-Perioden die Kohlensäure- und Wasserbildung, sowie die Gewichtsabnahme der Versuchstiere. Aus diesen Werten wird die Sauerstoffaufnahme berechnet. Dies Verfahren hat u. a. den Nachteil, dass für die verschiedenen 2-Stunden-Perioden sich immer nur ein Mittelwert ergibt. Eine eingehendere Analyse des Gasaustausches kann daher nicht durchgeführt werden. Temperaturmessungen konnten aus dem gleichen Grunde immer nur zwischen den verschiedenen 2-Stunden-Perioden vorgenommen werden. Die igelartig zusammengekauerte Haltung der Versuchstiere mit ihrem gesträubten Fell deutet LOENING zweifellos mit Recht als wärmesparende Massnahme. Der Verf. schliesst hieraus, dass die Herabsetzung der Körpertemperatur nicht ihre Ursache in einem erhöhten Wärmeverlust, sondern vielmehr in einer verminderten Wärmeproduktion hat. Letztere

wäre die Folge einer Herabsetzung der oxydativen Prozesse im Organismus. LOENING hält diese Verminderung der oxydativen Prozesse für die primäre Veränderung im chronischen anaphylaktischen Schock. Sie wäre ein weiteres Kennzeichen des Schockes, und würde durch spezifische allergische Veränderungen in den Zellen verursacht. Von der Möglichkeit, dass die Herabsetzung des Gasaustausches pulmonal bedingt sein könnte, nimmt der Verf. vollkommen Abstand.

HIRSCH und LESCHKE (1914) bestimmten mittels eines Respirationskalorimeters die Wärmeproduktion eines Hundes, der gegen Kaninchenserum sensibilisiert war, vor und nach der intravenösen Injektion von Kaninchenserum. Untersucht wurden nur Serummengen, die eine Temperatursteigerung hervorruften, leider aber keine grösseren Dosen, wie sie nach LESCHKE (1914) notwendig sind, um einen Abfall der Temperatur zu bewirken. In einigen Versuchen mit anaphylaktischem Fieber, wurde eine Herabsetzung des Stickstoffumsatzes und eine Verminderung der Wärmeproduktion gefunden. Die Versuche wurden über einen längeren Zeitraum ausgedehnt. Der Hund sass fast 24 Stunden hintereinander im Respirationskalorimeter, dieses wurde aber alle Stunde geöffnet, um die Rektaltemperatur des Tieres zu messen. Dies Vorgehen vermindert den Wert der Versuche erheblich, da hierdurch die Bestimmungen der vom Versuchstier abgegebenen Wärmemengen unsicher werden. Ausserdem wurde bei dem gleichen Tier ein Thymuspräparat (»Thymin« Poehl) ausprobiert, das der Hund täglich gerade bis zum 6. Tage vor dem deutlichsten Versuch erhielt. In wie weit dieses, der damaligen Zeit entsprechend, sehr unreine Präparat irgend einen Einfluss auf den Ausfall des Versuches hatte, ist natürlich nicht zu beurteilen.

An Meerschweinchen, die gegen Rinderserum sensibilisiert waren, bestimmten ABBERHALDEN und WERTHEIMER (1922) vor und nach intrakardialer Reinjektion des homologen Antigens die Kohlensäureproduktion in  $\frac{1}{2}$ -Stunden-Perioden, sowie nach der Methode von Warburg die Atmungsgrösse verschiedener Gewebe. Die Verfasser zeigten, dass die Kohlensäure-Produktion während des anaphylaktischen Schockes erheblich abnahm. Sie zogen hieraus den Schluss, dass der Gasaustausch herab-

gesetzt wäre. Es ist jedoch nicht möglich, sich aus derartigen Versuchen irgendeine sichere Vorstellung über den Sauerstoffverbrauch zu machen, da sowohl Veränderungen in der Grösse der Lungenventilation, wie auch solche im Milchsäuregehalt des Organismus erheblich die Menge der ausgeatmeten Kohlensäure beeinflussen. Man weiss, dass sich der Milchsäuregehalt des Blutes während des anaphylaktischen Schockes erhöht (McCULLOCH und O'NEILL, 1925). Untersuchungen über das Verhalten der Lungenventilation im anaphylaktischen Schock sind mir nicht bekannt, in dieser Arbeit aber wird gezeigt werden, dass sich die Lungenventilation während des experimentellen Asthmas erheblich verändert. Es ist daher wahrscheinlich, dass es auch im anaphylaktischen Schock zu entsprechenden Veränderungen der Lungenventilation kommt. Die Untersuchungen von ABDERHALDEN und WERTHEIMER geben also wenig Aufklärung über den gesamten Stoffwechsel an sich, sie sind aber um so interessanter, was die Gewebsatmung betrifft, da diese Autoren als erste eine Herabsetzung der Oxydationsgrösse in den Ge weben von Tieren nachwiesen, die im anaphylaktischen Schock starben oder getötet wurden. Alle untersuchten Gewebe, nämlich Oberschenkelmuskulatur, Herz, Leber und Gehirn, zeigten eine ungefähr gleich starke Abnahme der oxydativen Prozesse bis zu 50 %. Diese Veränderung wurde auch dann gefunden, wenn die Versuchstiere bereits nach 3 Minuten im anaphylaktischen Schock starben. Die Tatsache, dass die Atmungsgrösse schon nach so kurzdauerndem Schock abnahm, veranlasste ABDERHALDEN und WERTHEIMER zu dem Schluss, dass dies Verhalten nicht auf Veränderungen der zellulären Substanzen, die für den Ablauf der Oxydationsprozesse notwendig sind, beruhen könnte. Sie halten es vielmehr für wahrscheinlicher, dass durch die Reinkjection »Zustandsänderungen im Blut und darüber hinaus in sämtlichen Gewebszellen ausgelöst werden, die den Ablauf der Oxydationen stören«. Die Verfasser diskutieren als möglich, dass die Krämpfe während des anaphylaktischen Schockes und der Tod im Schock auf Störungen an den Zellen der wichtigsten Nervenzentren beruhen könnten. Die gleichen Autoren untersuchten auch die Kohlensäure-Produktion und die Gewebsatmung von Tauben (1922) vor und während eines anaphylak-

tischen Schockes, der durch Reinfektion nach intramuskulärer Sensibilisierung ausgelöst wurde. Auch in leichten Schockzuständen, die sich bei den Tauben vor allem in einer nachweisbaren Senkung der Körpertemperatur manifestierten, wurde eine Verminderung der Kohlensäure-Produktion, ein Temperaturabfall und eine Herabsetzung der oxydativen Prozesse in den Geweben gefunden. Letztere war am ausgeprägtesten im Gehirn, während die Geweboxydationen der Leber nur unwesentlich oder überhaupt nicht herabgesetzt waren. ABBERHALDEN und WERTHEIMER halten Untersuchungen des Gasaustausches und der Gewebsatmung für eine empfindliche Methode, um das Wesen der Anaphylaxie zu studieren, und für ein noch empfindlicheres Kriterium, um Veränderungen im Stoffwechsel der Zellen zu erkennen, als es die Beobachtung der Körpertemperatur darstellt.

An Mäusen, die durch intraperitoneale Injektionen gegen Pferdeserum sensibilisiert waren, bestimmte BENGELER (1931) den Sauerstoffverbrauch vor und nach intravenöser Injektion des homologen Antigens in die Schwanzvene. Bei kleinen Antigenmengen fand er eine Erhöhung der Sauerstoffaufnahme, bei grossen Antigendosen dagegen während mehrerer Stunden eine erhebliche Verminderung derselben. Diese Versuche wurden aber unter wenig physiologischen Bedingungen ausgeführt. Die Mäuse wurden in einen grossen Glaskolben (1500 cc) gesetzt, der mit einer röhrenförmigen Öffnung versehen war. Durch diese wurde der Schwanz der Maus herausgesteckt. Es ist nicht sehr ansprechend, wenn bei einer derartig gefesselten Maus der Sauerstoffverbrauch bestimmt wird. Außerdem dürften die Mäuse durch die intravenöse Injektion selbst erheblich erregt werden. Im Glaskolben war Lauge zur Absorption der ausgeatmeten Kohlensäure. Die Bestimmung der Sauerstoffaufnahme erfolgte manometrisch nach der Methode von Warburg. Korrekturen für die Veränderungen des Luftdruckes während der Versuchsdauer wurden nicht vorgenommen. Im Hinblick auf die kleine Blutmenge einer Maus von 18 gr Körpergewicht, stellt die intravenöse Injektion einer grösseren Serummenge (bis zu 0,7 cc) einen allzu grossen Eingriff dar, als dass man aus den Versuchsergebnissen irgendwelche sicheren Schlüsse

ziehen könnte. Zumindest die Erhöhung der Sauerstoffaufnahme, die BÜNGELER nach der Injektion kleiner Antigenmengen bei stark sensibilisierten Tieren oder von grösseren Antigenmengen bei ungenügend sensibilisierten Tieren fand, kann nicht als sicher angesehen werden, vor allem da der Verf. selbst zeigte (1933), dass die intravenöse Injektion an und für sich bereits eine erhebliche Steigerung der Oxydationen in den Geweben bewirkt. Für diese Reaktion führte Büngeler den Begriff des »Wasserfehlers« ein. Bereits früher wurden von WOLLMHEIM und BRANDT charakteristische »Wassereffekte« in anderer Beziehung (Veränderungen der Blutzusammensetzung usw.) nachgewiesen, die möglicherweise in ursächlichem Zusammenhang zu den von BÜNGELER gefundenen Reaktionen stehen. Die Herabsetzung der Sauerstoffaufnahme dagegen, die dieser Verf. nach grossen Antigenmengen bei stark sensibilisierten Tieren fand, kann zumindest in qualitativer Hinsicht als signifikativ angesehen werden. BÜNGELER untersuchte ferner an Meerschweinchen, Mäusen und Kaninchen, die im anaphylaktischen Schock getötet wurden, die Geweboxydationen der Lunge, Leber, Milz und Niere. Bei allen diesen Tierarten fand er im ausgesprochenen anaphylaktischen Schock, der durch grosse Antigenmengen an stark sensibilisierten Tieren (intraperitoneale und intravenöse Reinjektion) hervorgerufen wurde, eine Herabsetzung der Gewebsatmung. Bei kleinen Antigenmengen oder ungenügend sensibilisierten Tieren waren umgekehrt die Geweboxydationen erhöht. Auch hierzu ist zu bemerken, dass man es nicht als sicher ansehen kann, dass die Steigerung der Gewebsatmung wirklich durch die anaphylaktischen Reaktionen hervorgerufen wurden: denn die Injektion der gleichen Mengen von Antigen bewirkt bei nicht sensibilisierten Tieren ebenfalls eine Erhöhung des Gewebsstoffwechsels von ungefähr gleicher Grössenordnung. BÜNGELER meint durch seine Versuche »ein-deutig zu beweisen«, dass die Herabsetzung des Gasaustausches und die Temperatursenkung im anaphylaktischen Schock nicht die Folge der Atmungsbehinderung in den Lungen sind, sondern durch eine primäre Verminderung der oxydativen Prozesse in den Geweben zu Stande kommen, die von den Lungenveränderungen vollkommen unabhängig ist.

Auch der Stoffwechsel von sensibilisierten Geweben, denen in vitro das entsprechende Antigen zugesetzt wurde, wurde studiert.

KELLER (1927 und 1928) untersuchte mittels der Methode von Warburg die Wirkung eines Zusatzes von Tuberkulin zu den Geweben von normalen Meerschweinchen und zu denen von Tieren, die vorher tuberkulös infiziert waren. Er konnte weder im Sauerstoffverbrauch noch in der anaeroben Glykolyse irgend einen Unterschied in der Reaktion normaler und tuberkulöser Gewebe auf den Tuberkulinzusatz nachweisen. Ferner untersuchte er in einzelnen Versuchen die Wirkung des Zusatzes von Schweineserum zu Leberschnitten von Meerschweinchen, die gegen dieses Antigen sensibilisiert waren. Hierbei hatte der Sauerstoffverbrauch die gleiche Grösse, wie nach dem Zusatz von Homoserum.

BÜNGELER (1931) untersuchte in einzelnen Versuchen die Wirkung eines Zusatzes von Pferdeserum zu den Geweben von Kaninchen, die gegen dieses Antigen sensibilisiert waren. In manchen Versuchen fand er eine geringe Erhöhung der Gewebsoxydationen, in anderen eine leichte Herabsetzung (Methode von Warburg).

BOSTRÖM (1937) teilt mit, dass er nach dem Zusatz des homologen Antigens zu den Geweben von sensibilisierten Meerschweinchen, Kaninchen und Mäusen in den meisten Fällen bei grossen Antigendosen eine Herabsetzung, bei kleinen Antigenmengen dagegen eine Erhöhung der Gewebsatmung erhielt. In einer späteren Mitteilung (1938) konnten aber diese Versuchsergebnisse nicht reproduziert werden.

JENEY und Mitarbeiter (1940) untersuchten nach Zusatz des homologen Antigens in vitro die Gewebsoxydationen und die Milchsäurebildung einer Lebersuspension von Kaninchen, die gegen verschiedene Antigene (Rinderserum, Pferdeserum, Hühnereiweiss) sensibilisiert waren. Der Sauerstoffverbrauch der betreffenden Suspension war sehr niedrig. Man darf daher annehmen, dass die Oxydationssysteme sehr erheblich geschädigt waren. Nach Zusatz des spezifischen Antigens ergab sich eine Steigerung des Sauerstoffverbrauches um 9 bis 44 %. Der Unterschied war am ausgeprägtesten in den ersten 10-Minuten-

Perioden. Die Verfasser untersuchten ferner die Oxydationsintensität der Lebersuspension im Methylenblauversuch nach THUNBERG. Sie nehmen an, dass nach dem Zusatz des Antigens eine raschere Entfärbung (um 25 bis 30 %) stattfindet. Meiner Meinung nach können aber aus diesen Versuchen keine sicheren Schlüsse gezogen werden, da die Entfärbungszeiten stets zu kurz waren. In den Normalversuchen betrugen sie nur 2 bis 3 Minuten. Der Methylenblauzusatz (50 bis 100 γ) hätte grösser sein müssen. Die Verfasser untersuchten ferner die Milchsäurebildung in ihren Lebersuspensionen durch direkte Bestimmung der Milchsäure, nach verschieden langer Verweildauer im Warburg-Apparat, teils mit, teils ohne Zusatz von Antigen. Die Versuche waren ebenso angeordnet, wie die zur Untersuchung des Sauerstoffverbrauches. Bei Zusatz des spezifischen Antigens zu sensibilisierten Geweben stieg die Milchsäurebildung um 10 bis 17 % an, während der Antigen-Zusatz zu normalen Geweben keinerlei Wirkung hatte.

Bezüglich des Gasaustausches und der Gewebsatmung im anaphylaktischen Schock lässt sich zusammenfassend sagen: LOENING fand bei Meerschweinchen eine Herabsetzung der Sauerstoffaufnahme, BÜNGELER an Mäusen in schwerem anaphylaktischem Schock ebenfalls eine Verminderung der Sauerstoffaufnahme. HIRSCH und LESCHKE stellten eine Verkleinerung der Kalorienproduktion fest, wobei aber die möglichen Fehlerquellen der von diesen Autoren angewendeten Methode zu einer zurückhaltenden Bewertung ihrer Resultate zwingt. Eine Herabsetzung der Gewebsatmung des Meerschweinchens und der Taube im anaphylaktischen Schock wurde zuerst von ABDERHALDEN und WERTHEIMER nachgewiesen, und später von BÜNGELER bestätigt (Meerschweinchen, Mäuse, Kaninchen). Alle Verfasser nehmen an, dass die Verminderung des Gasaustausches und der Geweboxydationen auf primären Veränderungen in den Körperfzellen beruht, die für die anaphylaktische Reaktion charakteristisch und spezifisch sind. Bezüglich der Veränderung der Gewebsatmung, die bei Zusatz des spezifischen Antigens zu sensibilisierten Geweben *in vitro* eintritt, ist es nach den in der Literatur vorliegenden Angaben nicht möglich, sich eine einheitliche Auffassung über ihre Richtung zu bilden.

### 3. Asthma bronchiale.

Nach alten Arbeiten von LAENNEC (1826) und BIERMER (1870) beruht das Asthma bronchiale auf einem Krampf der Bronchialmuskulatur, nach TRAUBE (1878), WEBER (1872) und später auch CURSCHMANN (1882) aber auf einem Schwellungszustand der Bronchialschleimhaut. Wie dem auch sein mag, es stellt in jedem Fall eine Form der Stenoseatmung dar. Dies wurde u. a. auch durch Röntgenuntersuchungen mit Lipiodol als Kontrastmittel bestätigt, Diese Beobachtungen wurden im Anfall und im anfallsfreien Intervall von STEINBERG (1932) und von RIGLER und KOUCKY (1938) ausgeführt. Die Verfasser kamen zu dem Ergebnis, dass die Stenose initial durch einen Spasmus der Bronchialmuskulatur hervorgerufen wird. Später stellen durch die Schleimsekretion gebildete Pröpfe die wichtigste Ursache des asthmatischen Zustandes dar.

Mit einer kombinierten spirographischen und pneumographischen Methode untersuchten STAHELIN und SCHUTZE (1912) 4 Asthmatiker im Anfall, 2 von ihnen auch im Intervall. Bei 2 dieser Patienten wurde eine sichere Erhöhung der Lungenventilation während des Anfalles gefunden. Die Steigerung der Lungenventilation kam bei einem dieser Kranken durch eine Vergrösserung des Atemvolumens, bei dem anderen durch eine Erhöhung der Atemfrequenz zu Stande. Die Verfasser nehmen an, dass der Anstieg der Lungenventilation auf einer Überkompensation beruhe, indem der Organismus darnach strebe, das Atemhindernis zu überwinden. Inspirium wie Exspirium sind erschwert.

GRAFE (1923) gibt in seiner Übersicht über »Die pathologische Physiologie des Gesamtstoff- und Kraftwechsels bei der Ernährung des Menschen« an, dass er in nicht veröffentlichten Versuchen an Patienten mit Asthma bronchiale mit schwerer Dyspnoe stets erhöhte Werte für den Gasaustausch gefunden hätte, wenn auch dieser Anstieg nie mehr als 20 % gegenüber der Norm betrug. Die Erhöhung des Gaswechsels wurde aber nur dann gefunden, wenn die Kranken dyspnoisch waren. Nähere Angaben über die verwendete Methode macht der Autor nicht.

POLLITZER und STOLTZ (1925) untersuchten Asthma-Kranke täglich mit der Krogh'schen Methode. Sie beschreiben 4 Patienten. Fall 1 hatte in der ersten Zeit nächtliche Anfälle. Sein Grundumsatz war zu dieser Zeit um 50 % erhöht. Als die nächtlichen Asthma-Anfälle aufhörten, wurde der Grundumsatz normal. Fall 2 hatte keine Anfälle, sein Grundumsatz war normal. Fall 3 hatte anfangs leichte asthmatische Beschwerden. Sein Grundumsatz war zu dieser Zeit um 20 % erhöht. Nachdem der Patient beschwerdefrei geworden war, war sein Grundumsatz normal. Fall 4 war bei der Aufnahme beschwerdefrei, sein Grundumsatz normal. Eines Nachts bekam der Patient einen 2 Stunden dauernden Anfall. Am folgenden Morgen war die Atmung kaum mehr verändert, sein Grundumsatz-Wert war aber auf + 65 % erhöht. Dieser Anstieg verschwand wieder im Laufe einiger Tage. Eine so erhebliche Steigerung der Sauerstoffaufnahme im subasthmatischen Stadium, das durch Bronchitis, giemende und pfeifende Atemgeräusche und vertiefte Atmung gekennzeichnet ist, kann nach der Ansicht dieser Verfasser nicht allein auf der erhöhten Atemarbeit beruhen. Sie halten es daher für sicher, dass der Asthmaanfall mit einer zentral bedingten Stoffwechselsteigerung einhergeht. Im Intervall zwischen den Asthmaanfällen waren die Grundumsatz-Werte eher niedrig. Dies stimmt mit den Untersuchungen von TOPPER, GALUP, KERS und TELLER überein, während CRIER und MAC ELROY normale oder leicht erhöhte Werte fanden. Interessant ist die Beobachtung von POLLITZER und STOLTZ, dass bei Asthma-Patienten ohne Anfälle der Sauerstoffverbrauch unter Grundumsatz-Bedingungen in grossen periodischen Wellen schwankt.

WITTKOWER und PETOW (1927) untersuchten mittels des von FLEISCH konstruierten Pneumotachographen dyspnoische Zustände verschiedener Art. Von 3 Patienten im Asthma-Anfall hatten 2 eine vergrösserte Ventilation, nicht aber der dritte. Die gesteigerte Ventilation entstand in beiden Fällen durch eine Vertiefung der Atemzüge. Die Verfasser kommen zu dem Ergebnis, dass man im Asthmaanfall häufig, aber nicht immer, eine Steigerung der Ventilation findet. Sie halten eine schlechte

Durchmischung der Alveolarluft mit der Atmungsluft für die mögliche Ursache dieser Ventilations-Steigerung.

Nach GALUP (1928) haben Asthma-Patienten im Zusammenhang mit ihren Anfällen erhöhte Grundumsatz-Werte. Der Verf. untersuchte 3 Patienten mit schwerem Asthma während mehrerer Wochen, aber nur in einigermassen ruhigem Zustand (moderat dyspnea). Er fand folgende Werte: + 26 %, + 34 % und + 49 %. Der Verf. wendete eine Atemmaske an, bestimmte die Menge der Ausatmungsluft und analysierte diese. Im Gegensatz zu POLLITZER und STOLTZ nimmt dieser Verf. an, die Erhöhung des Grundumsatzes könnte auf der vergrösserten Atmungsarbeit, auf den Spasmen der Bronchialmuskulatur, auf der verstarkten Schleimsekretion und auf dem Ermündungsfaktor beruhen.

HERBST (1928) untersuchte Asthma-Patienten mit leichteren Atembeschwerden mittels der Douglas-Methode im Stehen. Die Lungenventilation pro Minute in der Ruhe war erhöht, während der Gasaustausch sich gleichzeitig innerhalb der normalen Grenzen hielt. Es liegen aber keine Untersuchungen über den Gasaustausch der gleichen Patienten im Anfalls-freien Intervall vor. Im Arbeitsversuch sind Patienten mit gleichzeitigen leichten asthmatischen Atemveränderungen nicht im Stande, ihre Lungenventilation ebenso zu steigern, wie die Gesunden. Die Sauerstoffaufnahme wird daher ungenügend.

KNIPPING und MONCRIEFF (1932) untersuchten mit Hilfe des Knipping'schen Apparates bei verschiedenen Arten von Dyspnoe das Ventilationsäquivalent, d. h. das Volumen Einatmungsluft in Litern, aus dem der Organismus 100 cc Sauerstoff aufnimmt. Durch die Anwendung des von ANTHONY eingeführten Begriffes des Ventilationsäquivalentes vermeidet man wenigstens teilweise die Abhängigkeit, in der die Ventilationsgrösse zu den Oxydationsprozessen steht. So bleibt beispielsweise das Äquivalent bei Muskelarbeit unverändert. Dies gilt nur für gesunde Versuchspersonen. Als Normalwert für das Ventilationsäquivalent wird von diesen Autoren 2,4 angegeben. 9 Patienten wurden während offenbar leichteren Asthmaanfällen untersucht. Von diesen hatten 3 ein erhöhtes Äquivalent (3,70 bis 5,65), während die übrigen 6 ein herabgesetztes Äquivalent hatten.

(1,62 bis 2,10). Erstere Gruppe bestand aus den unruhigen Patienten, die eine beschleunigte und erschwerte Atmung sowie ein vergrössertes Atem-Minutenvolumen hatten. Die Patienten der zweiten Gruppe zeigten keine psychische Unruhe, ihre Atmung war erschwert, langsam und vorsichtig, ihr Atem-Minutenvolumen vermindert. Nach der Injektion von Adrenalin veränderte sich das Ventilationsäquivalent in Richtung der Norm, die zu hohen Äquivalente wurden verkleinert, die zu niedrigen erhöht. Angaben über den Sauerstoffverbrauch in den einzelnen Fällen werden von den Verfassern nicht gemacht.

STRIECK und MARBLE (1936) bestimmten die Grösse des respiratorischen Gaswechsels an 6 Asthma-Kranken, die sich 24 Stunden in einem Grafe'schen Universalrespirationsapparat befanden. Die Sauerstoffaufnahme stieg unter diesen Bedingungen um 11 bis 42 % an, wobei von den Autoren nur ein Wert für 24 Stunden angegeben wird. Eine Veränderung des respiratorischen Quotienten wurde nicht gefunden. Die Verfasser nehmen an, dass die vermehrte Atmungs- und Herzarbeit die Ursache für die Steigerung des Sauerstoffverbrauches wäre. Letztere war am ausgeprägtesten, wenn ausser der Atmungsmuskulatur auch andere Muskelgruppen in Aktion traten, um durch eine Veränderung der Körperhaltung die Arbeit der Atmungsmuskulatur zu erleichtern.

ROELSEN (1937) fand bei 17 Asthma-Kranken, die in einigermassen anfallsfreiem Zustand untersucht wurden, in 55 Bestimmungen mit der Douglas-Methode nur 8 mal eine wirkliche Hyperventilation (ohne gleichzeitige Erhöhung des respiratorischen Quotienten). Bei Letzteren war das durchschnittliche Atem-Minutenvolumen 10,35 Liter (reduziert auf 760 mm Druck, 0°, Trockenheit). Der Normalwert der Lungenventilation pro Minute war bei gesunden Versuchspersonen 6,18 Liter. Der Durchschnittswert des Atemminutenvolumens aus allen 55 Bestimmungen an Asthma-Patienten war 8,28 Liter. Vergleicht man die gefundenen Ventilations-Werte mit dem klinischen Zustand der Kranken, so findet man, nach ROELSEN, häufig einen Zusammenhang in dem Sinne, dass eine Vergrösserung der Atem-Minutenvolumina bei dyspnoischen Patienten mit vertiefter Atmung vorkommt. Die Sauerstoffaufnahme war bei

diesen Patienten erhöht und lag im Mittel um 21 % über den basalen Werten. Der respiratorische Quotient war im Mittel 0,81 (0,74 bis 1,02). Die Ursache dieser häufig vorkommenden Erhöhung der Basal-Werte ist, nach Roelsex, darin zu sehen, dass die Kranken während der Untersuchung keine basalen Versuchsbedingungen aufrechterhalten können. Sie sind dyspnoisch, husten oft und liegen nicht so still, wie Gesunde. Es muss aber als bemerkenswert hervorgehoben werden, dass Patient Nr. 5, der dreimal untersucht wurde, die niedrigste Ventilation pro Minute und die kleinste Sauerstoffaufnahme in einem Zustand zeigte, als seine Atmung deutlich asthmatisch war. Bei späteren Untersuchungen mit ruhigerer Atmung war sein Sauerstoffverbrauch grösser. Roelsex zeigte ferner mittels fraktionierter Alveolarluft-Analysen, dass bei Asthma- und Emphysem-Patienten, die in einigermassen anfallsfreien Zuständen untersucht wurden, die Ventilation der verschiedenen Lungenabschnitte erheblich ungleichmässiger ist, als unter normalen Verhältnissen. Untersuchungen im Asthma-Anfall selbst wurden nicht durchgeführt. Man kann aber, nach Roelsex, als sicher annehmen, dass im Anfall die Ventilation in grossen Teilen der Lungen stark herabgesetzt ist.

Zusammenfassend lässt sich also sagen, dass, nach den in der Literatur vorliegenden Angaben, der Sauerstoffverbrauch bei Asthma-Patienten im Zusammenhang mit dem Anfall erhöht ist. Was die Lungenventilation pro Minute betrifft, so sind die Angaben verschieden. Die meisten Autoren (STAERELIN und SCHÜTZE, WITTKOWER und PETOW, ROELSEX) fanden erhöhte Werte, während KNIPPING und MONCRIEFF im Vergleich mit der Lungenventilation in anfallsfreien Zeiten teils zu hohe, teils zu niedrige Werte beobachteten.

#### 4. Das Verhalten der Temperatur.

SENATOR (1868) unterscheidet zwei Arten von Atembehinderung, nämlich eine Gruppe, bei der das Hindernis leichterer Art ist und vom Organismus durch eine kräftige Atmung kompensiert werden kann, und eine zweite Gruppe, bei der das Hinder-

nis so schwer ist, dass es nicht kompensiert werden kann. Letzteren Zustand nennt SENATOR Atmungsinsuffizienz. Er ist gekennzeichnet durch Cyanose und Absinken der Temperatur. SENATOR rief experimentell Atemstörungen auf verschiedene Weise hervor: durch Anlegen einer Binde um den Thorax, durch Anlage einer Ligatur, die dann zusammengezogen wurde, um die Trachea, durch Einspritzung von Öl in die Trachea und durch Einlegen eines Ballons in die Pleurahöhle. Er benutzte für seine Versuche Kaninchen, Hunde und Katzen. Bei leichterer Behinderung der Atmung konnten die Versuche über mehrere Tage ausgedehnt werden, bei schwererer Behinderung nur einige Stunden bis zu einem Tag. Bei leichteren Hindernissen fand SENATOR meist eine Erhöhung der Körpertemperatur bis zu 1°. Er nahm an, dass der Temperaturanstieg auf der erhöhten Leistung der normalen und accessorischen Atemmuskulatur und der vergrösserten Herzarbeit beruhe. Bei schwererer Behinderung der Atmung konnte die Körpertemperatur bis zu 6° während drei Stunden sinken. Die Ursache hierfür ist, nach SENATOR, in einer Herabsetzung der Oxydationsprozesse und in einer Erhöhung der Wärmeabgabe durch die Haut zu sehen. Die Versuchstiere waren in diesem Fall cyanotisch und hinfällig. Beseitigte man das Hindernis, so erholten sie sich allmählich.

In seinen weiter oben besprochenen Versuchen fand KÖHLER (1877) am Tage nach der Anlage einer Atemstenose bei vier von insgesamt fünf untersuchten Kaninchen eine Temperatursteigerung. Diese schwankte zwischen 0,3 und 1,4°. Dieser Temperaturanstieg blieb mehrere Tage bis zu drei Wochen bestehen. Einige Tage, bevor die Versuchstiere starben, begann die Temperatur zu sinken. Bei einem Kaninchen fiel die Temperatur von Anfang an, und blieb während der folgenden Woche 0,3 Grad niedriger, als vor Anlage der Stenose. Die Temperatursteigerung erklärt KÖHLER auf die gleiche Weise, wie SENATOR, während er bezüglich der Temperatursenkungen annimmt, dass sie auf hinzukommenden Kreislauftörungen beruhten.

PFEIFFER (1909) beschrieb als erster die Temperatursenkung im anaphylaktischen Schock des Meerschweinchens. Er hielt diese für ein neues charakteristisches Kennzeichen des ana-

phylaktischen Schocks, das von den anderen Symptomen unabhängig wäre und regelmässiger als diese auftrete. PFEIFFER bezeichnete die Temperatursenkung als den »anaphylaktischen Temperatursturz«. Wegen seiner strengen Spezifität und seiner grossen Empfindlichkeit ist, nach PFEIFFER, der Nachweis des anaphylaktischen Temperatursturzes zur Benutzung in forensischen Fällen geeignet. Der Verf. zeigte, dass dieser anaphylaktische »Temperatursturz« nicht früher, als 14 Tage nach der Sensibilisierung, ausgelöst werden kann. Nach seiner Auslösung folgt eine Periode von mehreren Wochen, während der es unmöglich ist, ihn hervorzurufen. Dies wäre nach PFEIFFER »eine neue Äusserungsform der Anti-Anaphylaxie«. Damit ist deutlich ausgedrückt, dass PFEIFFER den Temperatursturz nicht für eine Folgeerscheinung des Auftretens der übrigen Symptome des anaphylaktischen Schockes hält, sondern für ein selbstständiges Parallelsymptom zu diesen. Der Abfall der Temperatur lässt sich am besten durch intraperitoneale Reinkjection hervorrufen.

Bereits früher wurde LOENING's (1911) grundlegende Arbeit erwähnt, in der nachgewiesen wurde, dass leichte anaphylaktische Symptome mit einer Temperatursteigerung einhergehen, während bei einem schweren Schock die Temperatur sinkt.

FRIEDBERGER und MITA (1911) führten an Meerschweinchen, die gegen Hammelserum sensibilisiert waren, eingehende Untersuchungen über die Einwirkung verschieden grosser Dosen des homologen Antigenes auf die Temperatur der Versuchstiere durch. Sie konnten dabei mit abnehmenden Antigenmengen vier verschiedene Grenzwerte feststellen, nämlich: 1.) Die Grenze des Temperatursturzes, d. h. die schwächste Dosis, die gerade noch einen Temperaturabfall bewirkt. 2.) »Die obere Konstanzgrenze«. Hiermit werden die Antigendosen bezeichnet, die zwischen den temperatursenkenden und temperatursteigernden Mengen liegen, und die selbst die Temperatur nicht verändern. Die Bezeichnung »Grenze« ist hier nicht ganz zutreffend, und deshalb in Anführungsstriche gesetzt. 3.) Die Fiebergrenze, d. h. die schwächste Dosis, die Fieber hervorruft. 4.) Die untere Konstanzgrenze; Antigenmengen unterhalb dieser Dosis rufen keine Temperaturveränderungen hervor. Welche

Reaktion nach Reinkjection auftritt, ist nach FRIEDBERGER und MITA ausschliesslich eine Dosierungsfrage. Je nach der Grösse der Dosis können drei verschiedene Erscheinungsformen der Anaphylaxie unterschieden werden, nämlich der mehr oder minder akut verlaufende und tödliche Schock nach den grossen Dosen, der psychogene Verlauf mit Temperatursenkung nach den mittelgrossen Dosen, und schliesslich der pyogene Verlauf nach kleinen Dosen. Nach einem Gedankengang DOERR's stellen die anaphylaktischen Reaktionen nur eine Steigerung normaler physiologischer Vorgänge dar. Von dieser Vorstellung und der Tatsache ausgehend, dass jedes beliebige artfremde Eiweiss, in genügend grossen Mengen injiziert, ein Meerschweinchen unter Symptomen, die dem anaphylaktischen Schock vollkommen gleichen, zu töten im Stande ist, untersuchten FRIEDBERGER und MITA auch die Wirkung verschieden grosser Mengen von Hammelserum auf nicht vorbehandelte Meerschweinchen. Sie konnten hierbei, in vollkommener Übereinstimmung mit dem Verhalten der sensibilisierten Tiere, ebenfalls die vier verschiedenen Grenzdosen feststellen. Ein Unterschied zwischen den sensibilisierten und den nicht vorbehandelten Tieren besteht nur darin, dass letztere weit grössere Mengen von Antigen zur Auslösung der entsprechenden Effekte brauchen. Das Verhältnis der entsprechenden Grenzdosen für normale und sensibilisierte Tiere nannten FRIEDBERGER und MITA den anaphylaktischen Index. Dieser kann so hohe Werte wie  $10^6$  erreichen.

LE SCHKE (1914) wies nach, dass man auch beim Hund und beim Kaninchen die vier von FRIEDBERGER und MITA gefundenen Grenzdosen austitrieren kann. Der Verf. weist besonders auf die eigentümlichen Verhältnisse in der anaphylaktischen Reaktion bei der »oberen Konstanzgrenze« hin. Als Beispiel führt er u. a. die Grenzdosen für einen Hund von 8 kg Gewicht bei intravenöser Injektion des homologen Antigenes (Homoserum) an. Es waren folgende Mengen: untere Konstanzgrenze 0,5 cc, Grenze für Fieber 1,5 cc, obere Konstanzgrenze 15 cc, Grenze des Temperatursturzes 30 cc. Nach der Dosis, die der oberen Konstanzgrenze entsprach, hatte der Hund einen schweren anaphylaktischen Schock, lag auf der Seite und hatte Krämpfe,

Erbrechen und Diarrhoe. Trotzdem war die Körpertemperatur konstant.

Nach CIRRON und LESCHKE (1913), sowie HASHIMOTO (1915) »verhindert die operative Ausschaltung des Zwischenhirnes das anaphylaktische Fieber«. Wie HASHIMOTO angibt, kann man durch Injektion des homologen Antigenes direkt in die Gegend der temperaturregulierenden Zentren bei sensibilisierten Tieren, je nach der Grösse der angewandten Dosen, Temperaturfall oder Temperatursteigerung auslösen. Die hierzu notwendigen Dosen sind kleiner als die, die man bei intravenöser Reinjektion anwenden muss. Dies gelingt nicht bei nicht sensibilisierten Tieren. HASHIMOTO nimmt an, dass das Wärmezentrum selbst sensibilisiert wäre und bei dem Kontakt mit dem Antigen mit Fieber (Reizungsssymptom) oder mit einem Temperatursturz (Lähmungs-symptom) reagiere, HASHIMOTO gibt aber selbst an, dass nach intracerebraler Injektion stets schwere anaphylaktische Symptome mit Atemnot und Krämpfen auftreten. Diese Symptome können jedoch kaum in irgend eine Beziehung zu den temperaturregulierenden Zentren gebracht werden, sondern lassen sich nur als echte anaphylaktische Reaktion deuten. DOERR (1929) weist auch darauf hin, dass es eigentlich wäre, wenn nur das Wärmezentrum und nicht gleichzeitig irgendwelche anderen Zentren, z. B. die Augenmuskelkerne, sensibilisiert sein sollten. Dieser Verf. betont, dass man einen anaphylaktischen Schock durch intracerebrale Injektion ebenso gut auslösen kann, wie durch einen der anderen Injektionswege.

STORM VAN LEUWEN, BIEN und VAREKAMP (1924) untersuchten u. a. das Verhalten der Körpertemperatur bei Meerschweinchen, bei denen durch Inhalation milbenhaltigen Kornes anaphylaktische Reaktionen hervorgerufen wurden. Bei leichten Symptomen stieg die Temperatur um 0,2 bis 0,3 Grad (manchmal auch um 0,5 bis 1,5 Grad) nach 3 bis 4 stündigem Aufenthalt in einer Atmosphäre, die das Antigen enthielt. Bei den Tieren, bei denen schwerere anaphylaktische Symptome mit erschwerter Atmung auftraten, sank dagegen die Temperatur, zuweilen sogar bis auf 30°.

DOERR (1929) betont, dass die Anschauungen über die Ursache der Temperaturveränderungen weit auseinandergehen. Er

schreibt: »Im allgemeinen herrscht die Tendenz, Fieber und Temperatursturz als Prozesse von identischem, nur dem Grade nach verschiedenem Mechanismus, und zwar als Reizungen oder Lähmungen der thermoregulatorischen Centralapparate aufzufassen und als Träger dieser Effekte Substanzen anzunehmen, welche in kleinen Gaben erregend, in grossen Dosen funktionshemmend wirken».

Zusammenfassend lässt sich über die Temperaturveränderungen bei mechanischer Stenoseatmung und im anaphylaktischen Schock sagen: SENATOR und, in gewissen Grade auch KOHLER zeigten, dass es bei Versuchstieren mit Behinderung der Atmung zu Veränderungen der Körpertemperatur kommt, und zwar so, dass bei leichteren Hindernissen die Temperatur steigt, während sie bei hochgradiger Behinderung der Atmung sinkt. PFEIFFER wies als erster den Temperatursturz im anaphylaktischen Schock nach; dies wurde später von FRIEDBERGER und MITA bestätigt, die ausserdem fanden, dass es nur nach grossen Antigendosen zu dem Temperatursturz kommt, während kleine Antigenmengen umgekehrt die Temperatur erhöhen. Die meisten Forscher (FRIEDBERGER und MITA, CITRON und LESCHKE u. a.) nehmen an, dass die Temperaturveränderungen bei den anaphylaktischen Reaktionen durch eine Einwirkung auf die temperaturregulierenden Zentra verursacht werden. Gewisse andere Autoren dagegen (LOENING, DOMAGK) halten die Temperaturveränderungen für sekundärer Art.

### 5. Zusammenfassende Literaturübersicht.

Der mechanischen Stenoseatmung, dem anaphylaktischen Schock und dem Asthma bronchiale ist gemeinsam, dass bei allen diesen Zuständen die Luftpassage von und zu den Lungen, wenn auch in verschiedener Weise, behindert ist. Dies Atemhindernis dürfte die Ursache der Dyspnoe sein, oder mindestens der wichtigste Faktor für ihre Entstehung. Man darf daher mit Recht erwarten, dass bei allen drei Zuständen Veränderungen der Lungenventilation von prinzipiell gleicher Art auftreten werden, in dem Bestreben des Organismus, die Atembehinderung zu kom-

pensieren, solange dies noch möglich ist, und damit den adäquaten Sauerstoffbedarf des Organismus zu sichern. Man darf ferner zweifellos erwarten, dass bei allen drei Zuständen die Lungenventilation und die Sauerstoffsaufnahme sich in prinzipiell gleicher Weise verändern werden, wenn die Atembehinderung so stark wird, dass es dem Organismus nicht möglich ist, sie zu kompensieren, und es daher zu einem Insuffizienz-Zustand der Atmung kommt.

Angaben über das Verhalten der Lungenventilation im anaphylaktischen Schock liegen meines Wissens in der Literatur nicht vor. Vergleicht man die zur Verfügung stehenden Angaben über die Lungenventilation bei mechanischer Stenoseatmung und bei Asthma bronchiale, so findet man, dass bei beiden Zustandsbildern teils von einer Vergrösserung, teils von einer Verkleinerung der Lungenventilation berichtet wird. Für die mechanische Stenoseatmung wird von mehreren Verfassern (LIPFELT, MATTHES) angegeben, dass die Lungenventilation bei leichterer Stenosierung erhöht, bei schwererer herabgesetzt wird.

Vergleicht man die Angaben über die Sauerstoffsaufnahme bei diesen drei Zuständen, so sieht man, dass diese Atmungsgrösse bei leichter mechanischer Stenoseatmung (MATTHES) und bei geringgradigen anaphylaktischen Symptomen (LOENING) erhöht, dagegen bei schwererer mechanischer Stenoseatmung (MATTHES) und bei ausgesprochenen anaphylaktischen Symptomen (LOENING, BÜNGELER) herabgesetzt ist. Bei Asthma bronchiale ist die Sauerstoffsaufnahme nach allen Untersuchungen, die in der Literatur vorliegen, erhöht (GRAFE, POLLITZER und STOLTZ, GALUP, STRIECK und MARBLE, ROELSEN).

Die Untersuchungen über den Sauerstoffverbrauch bei Asthma bronchiale wurden in der Regel bei leichteren asthmatischen Beschwerden oder nach dem Abklingen eines schweren Anfalles durchgeführt. Man muss sich daher fragen, ob nicht der Unterschied im Verhalten des Sauerstoffverbrauches, wie er nach den Angaben der Literatur zwischen der mechanischen Stenoseatmung und dem anaphylaktischen Schock einerseits, dem Asthma bronchiale andererseits zu konstatieren ist, darauf beruhen könnte, dass die Untersuchungen bei den ersten beiden Zuständen unter der bestehenden Atmungsbehinderung, beim Asthma

bronchiale aber erst einige Zeit nach einem schweren Anfall erfolgten. Für die Beurteilung dieser Frage ist es wesentlich, festzustellen, wie sich der Sauerstoffverbrauch in der Nachperiode nach einer mechanischen Stenoseatmung und nach einem anaphylaktischen Schock verhält.

Was die mechanische Stenoseatmung betrifft, so liegen hierüber in der Literatur keine Angaben vor. Nach einem anaphylaktischen Schock steigt der Sauerstoffverbrauch, nach den Untersuchungen von LOENING, wieder zu normalen Werten an. Eine Erhöhung der Sauerstoffaufnahme über die Normalwerte ist in LOENINGS Versuchen nicht zu konstatieren. Es ist aber nicht möglich, sich aus LOENINGS Versuchen eine sichere Vorstellung von dem Sauerstoffverbrauch in der Nachperiode nach einem anaphylaktischen Schock zu bilden, da dieser Autor nur einen Wert für eine 2-Stunden-Periode angibt. In dieser Zeit können natürlich erhebliche Veränderungen in der Sauerstoffaufnahme erfolgt sein. Man darf aber mit Recht vermuten, dass die Sauerstoffaufnahme in der Nachperiode nach einer schweren mechanischen Stenoseatmung wie nach einem ausgesprochenen anaphylaktischen Symptomenkomplex mit herabgesetztem Sauerstoffverbrauch über die Normalwerte hinaus erhöht ist. Nach Untersuchungen von MOORE und BINGER sinkt während der mechanischen Stenoseatmung die Sauerstoffsättigung des arteriellen Blutes um 50 %. Im anaphylaktischen Schock nimmt, nach MC. CULLOGH und O'NEILL, der Milchsäure-Gehalt des Blutes zu, das pH des Blutes kann, nach HIRSCH und WILLIAMS, um mehrere Einheiten in der ersten Dezimale sinken. Gleichzeitig vermindert sich die Alkalireserve. All dies spricht dafür, dass während schwerer mechanischer Stenoseatmung und im schweren anaphylaktischen Schock eine Sauerstoffschuld entsteht. Man hat daher alle Veranlassung, in der Nachperiode nach diesen Arten von Stenoseatmung eine über die Norm hinausgehende Steigerung der Sauerstoffaufnahme zu erwarten. Gemäss dieser Betrachtungsweise und unter der Voraussetzung, dass auch für das Asthma bronchiale entsprechende Bedingungen gelten könnten, wäre es durchaus möglich, dass die Steigerung des Sauerstoffverbrauches, die man bei Asthma bronchiale beobachtet hat, auf der Durchführung der Bestimmungen gerade in dieser Nach-

periode beruhen, in der die vorher aufgehäufte Sauerstoffschuld zurückerstattet wird. Für die Berechtigung dieser Auffassung sprechen gewisse Angaben in der Literatur die schon früher erwähnt wurden. Unter diesen möchte ich vor allem noch einmal auf zwei Beobachtungen hinweisen. POLITZER und STOLTZ berichten von einem Patienten, der bei der Aufnahme in das Krankenhaus einen normalen Sauerstoffverbrauch hatte. Nachts bekam der Kranke einen Asthma-Anfall von 2-stündiger Dauer. Am folgenden Morgen war die Sauerstoffaufnahme um 65 % erhöht. Es liegt nahe, anzunehmen, dass in diesem Fall die vermehrte Sauerstoffaufnahme auf der Rückerstattung einer Sauerstoffschuld beruhte. Unter den Asthma-Patienten, die ROELSEN untersuchte, gab es nur einen, der zur Zeit der Untersuchung eine deutliche asthmatische Atmung hatte. Dieser Kranke hatte damals eine Sauerstoffaufnahme von + 2 %. Bei einer späteren Untersuchung war die Sauerstoffaufnahme dieses Patienten grösser. Dass die Sauerstoffaufnahme bei Asthma bronchiale gestört ist, wurde von MEAKINS (1921) nachgewiesen. Dieser zeigte, dass bei erschwerter Atmung die arterielle Sauerstoffsättigung um etwa 10 % herabgesetzt ist. Unter der Einatmung von Sauerstoff wurden diese Werte wieder normal.

Temperaturveränderungen, die in einem näheren ursächlichen Zusammenhang zu den verschiedenen Zuständen mit Atmungsbehinderung standen, wurden für die mechanische Stenoseatmung und für den anaphylaktischen Schock beschrieben. Bei Asthma bronchiale wurde, meines Wissens, über keine derartigen Temperaturveränderungen berichtet. Dagegen gibt es mehrere Angaben in der Literatur über den günstigen Einfluss des Fiebers, sei es eines spontanen, sei es eines künstlich hervorgerufenen, auf den Verlauf beim Asthma bronchiale (z. B. BEZANCON und JACQUELIN, 1931).

Bei mechanischer Stenoseatmung (SENATOR, KOHLER) wie auch im anaphylaktischen Schock (PFEIFFER, FRIEDBERGER und MITA u. a.) wurden Temperatursteigerungen und Temperatursenkungen beobachtet. SENATOR fand bei leichteren Symptomen eine Temperatursteigerung und nahm an, dass diese die Folge der vergrösserten Atmungs- und Herzarbeit wäre. Bei schwererer Stenoseatmung, die vom Organismus nicht kompensiert werden

konnte, fand SENATOR umgekehrt eine erhebliche Herabsetzung der Körpertemperatur. Er nahm an, dass diese teils auf einer erhöhten Wärmeabgabe, teils auf einer Herabsetzung der oxydativen Prozesse in den Zellen beruhte. Die Temperaturveränderungen, die man im anaphylaktischen Schock beobachtete, hielt man im allgemeinen für die Folge von Einwirkungen auf die temperaturregulierenden Zentren (FRIEDBERGER und MITA, CITRON und LESCHKE, HASHIMOTO). Hierbei sollten kleine Antigendosen, die die Temperatur erhöhen, die betreffenden Zentra reizen, während grössere Antigenmengen, die die Temperatur herabsetzen, sie lähmen würden.

Nach den Angaben der Literatur würden also die Temperaturveränderungen bei der mechanischen Stenoseatmung einerseits, im anaphylaktischen Schock andererseits auf ganz verschiedenen Ursachen beruhen.

Es erscheint mir aber wenig wahrscheinlich, dass die Temperaturveränderungen bei einem so komplizierten Symptomenkomplex, wie es der anaphylaktische Schock ist, so einheitlich bedingt wären, wie man im allgemeinen annimmt. In den oben erwähnten Versuchen von SENATOR und KÖHLER wurde mit aller wünschenswerten Deutlichkeit gezeigt, dass man bei mechanischer Behinderung der Atmung eine Temperatursteigerung erhält, wenn das Hindernis leicht ist, eine Temperatursenkung dagegen, wenn die Behinderung stark ist. Eines der typischsten Symptome des anaphylaktischen Schockes ist gerade die Erschwerung der Atmung, vor allem beim Meerschweinchen, aber mehr oder minder deutlich auch bei anderen Tierarten. Die meisten Forscher aber, die sich für die Temperaturveränderungen bei der Anaphylaxie interessierten, studierten diese wie einen isolierten Vorgang. Sie berücksichtigten, meiner Meinung nach, nicht genügend die Einwirkungen auf die Temperatur, die andere anaphylaktische Symptome notwendigerweise mit sich bringen mussten, obgleich doch deutlich betont wurde, dass man bei einer leichten Erhöhung der Antigendosen, die einen Temperatursturz auslösen, in den Bereich der letalen Dosen kommt. Dass aber beim Meerschweinchen die unmittelbare Todesursache im Schock in einer Erstickung durch hochgradige Einengung des Bronchiallumens besteht, darüber sind sich fast alle Forscher einig. Es

gibt allerdings einige Autoren, die die Temperaturveränderungen als sekundäres Phänomen betrachten. So hält LOEING, dessen Untersuchungen oben erwähnt wurden, den Temperatursturz für die Folge der primären Herabsetzung der oxydativen Prozesse im Organismus, die ihrerseits durch die spezifischen anaphylaktischen Veränderungen ausgelöst würden. DOMAGK (1925) nahm an, dass histologische Veränderungen in den Lungen, vor allem an den Kapillaren in den Alveolarsepten, die teilweise für Blut undurchgängig würden, die primäre Ursache für die Herabsetzung der oxydativen Prozesse und die Temperatursenkung wäre, wie sie von anderen Forschern nachgewiesen wurden.

DOERR (1929) betont, dass man auf gewisse Schwierigkeiten stösst, wenn man die obere Konstanzgrenze allein durch die Theorie erklären will, dass kleine Antigendosen reizend, grosse lähmend auf die temperaturregulierenden Zentren wirken. An der oberen Konstanzgrenze müssten sich reizende und lähmende Effekte gegenseitig aufheben. Wenn auch diese Erklärung theoretisch möglich wäre, kann man, meiner Meinung nach, das Verhalten der Temperatur an der oberen Konstanzgrenze weit leichter so erklären: Bei den fraglichen Antigendosen wird die Atmung gerade so weit behindert, dass die Sauerstoffaufnahme ungenügend ist. Dieser Vorgang senkt die Temperatur und wirkt der vorher bestehenden Temperatursteigerung entgegen, die ihrerseits teilweise durch Reizung der temperaturregulierenden Zentren ausgelöst sein könnte.

## 6. Fragestellung.

Um zu einer einheitlicheren Auffassung über die Sauerstoffaufnahme und das Verhalten der Körpertemperatur bei den verschiedenen Formen der Stenosatmung zu gelangen, kann man meines Erachtens auf Grund der Ausführungen in Abschnitt 5 die folgende Arbeitshypothese aufstellen: Das unterschiedliche Verhalten der Sauerstoffaufnahme, das nach den Angaben der Literatur bei schwerer mechanischer Stenosatmung und im anaphylaktischen Schock einerseits, bei schwerem Asthma bronchiale andererseits festzustellen ist, hat seinen Grund darin, dass

bei den beiden erstgenannten Zuständen die Bestimmungen des Gaswechsels unter der noch bestehenden Atmungsbehinderung, beim Asthma bronchiale aber erst dann ausgeführt wurden, wenn die Atmungsbeschwerden schon zum grössten Teil abgeklungen waren, also in einer Periode, in der die Rückerstattung der vorher eingegangenen Sauerstoffschuld erfolgte. Teilweise wurde auch der Sauerstoffverbrauch mit nur einem einzigen Wert während einer so langen Zeitperiode bestimmt, dass während dessen grössere Variationen dieses Wertes möglich waren, die sich der Beobachtung entziehen konnten. Man hat ferner gute Gründe, als Arbeitshypothese aufzustellen, dass die Temperaturveränderungen bei der mechanischen Stenoseatmung und im anaphylaktischen Schock zum Teil auf die gleiche Art und Weise ausgelöst werden, nämlich insoweit, als die Temperatursteigerung, die bei leichteren Symptomen zu beobachten ist, u. a. auf der erhöhten Atmungs- und Herzarbeit beruht, während die Temperatursenkung bei den schwereren Symptomenbildern vor allem eine Folge des Sauerstoffmangels ist. Hierbei ist aber zu betonen, dass die Temperaturveränderungen im anaphylaktischen Schock sicher von weit komplizierterer Natur sind, als die bei der mechanischen Stenoseatmung; denn beim Schock wird bekanntlich artfremdes Eiweiss in den Organismus eingeführt und löst in ihm seine verschiedenen Wirkungen, u. a. auch die auf die Temperatur, aus.

Um im einzelnen festzustellen, inwieweit die Gedankengänge der oben dargestellten Arbeitshypothesen berechtigt sind, erschien es richtig *eine* Art von Stenoseatmung so genau wie möglich zu untersuchen. In der hier vorgelegten Arbeit wurden mit möglichst einwandfreier Methode die Lungenventilation, die Sauerstoffaufnahme und das Verhalten der Körpertemperatur bei einer experimentell ausgelösten Stenoseatmung untersucht. Um die Verhältnisse richtig beurteilen zu können, ist es vor allem notwendig, die Beobachtungen auf die Zeit vor, während und nach der Stenoseatmung auszudehnen. Ferner musste die Sauerstoffaufnahme in der Vorperiode, während der Stenoseatmung und in der Nachperiode kontinuierlich bestimmt werden. Dies geschah in früheren Versuchen nicht. Aber nur so können die Veränderungen in der Sauerstoffaufnahme, die unter der

Stenoseatmung auftreten, näher analysiert werden. Für die Bestimmung der Grösse der Lungenventilation war mir eine kontinuierliche Registrierung nicht möglich. Stattdessen wurden jeweils mehrere Bestimmungen vor, während und nach der Stenoseatmung ausgeführt. Wollte man die Temperaturschwankungen während der Stenoseatmung unter besonderer Berücksichtigung der Beziehungen dieser Temperaturvariationen zu der Grösse der Sauerstoffaufnahme untersuchen, so war es ebenfalls notwendig, die Temperatur kontinuierlich zu registrieren, ohne hierdurch das Versuchstier zu beunruhigen. Um die Schwankungen der Körpertemperatur richtig beurteilen zu können, musste ausserdem die Hauttemperatur bestimmt werden; denn nur auf diese Weise kann man sich eine richtige Vorstellung von der Wärmeabgabe machen. Soweit mir bekannt, wurden derartige Messungen bei der Stenoseatmung bisher nicht durchgeführt. Die einzige hierfür adäquate Methode ist das thermoelektrische Verfahren. In allen früheren Untersuchungen über das Verhalten der Temperatur bei der Stenoseatmung wurde die Rektaltemperatur gemessen, indem zu verschiedenen Zeiten ein Thermometer in das Rectum der Versuchstiere eingeführt wurde. Hierdurch mussten diese aber irritiert werden, vor allem während der Stenoseatmung. Schliesslich war es für diese Untersuchungen notwendig, die Stenoseatmung auf eine für das Versuchstier möglichst schonende Art zu bewirken. In den früheren Untersuchungen über die Stenoseatmung im anaphylaktischen Zustand wurde dieser stets durch Injektion ausgelöst. Es lässt sich aber nur sehr schwer beurteilen, inwieweit die Versuchstiere durch diesen Eingriff irritiert werden. So betonen beispielsweise FRIEDBERGER und MITA (1911), dass die Fesselung der Tiere, die nach diesen Autoren für eine quantitative Injektion beim Meerschweinchen nötig ist, eine Temperatursenkung von mehr als 1 Grad bewirkt. Da es aber für diese Autoren allein auf den Vergleich der Wirkung verschieden starker Antigendosen ankam, massen sie diesem Verhalten keine grössere Bedeutung zu. Um u. a. diese Schwierigkeiten zu vermeiden, die Injektionen mit sich bringen können, untersuchte ich die Tiere im experimentellen Asthma, wobei die Atembehinderung durch Inhalation ausgelöst wurde. Auf diese Weise wird eine

Stenoseatmung bewirkt, ohne dass das Versuchstier von aussen her irgendwie irritiert wird. Dieses sitzt während der ganzen Versuchsdauer ruhig im Respirationsapparat. Dass Meerschweinchen für diese Versuche gewählt wurden, hat seinen Grund teils darin, dass sich bei diesen Versuchstieren anaphylaktische Reaktionen am leichtesten auslösen lassen, teils darin, dass sie für Untersuchungen der Sauerstoffaufnahme besonders geeignet sind, da ihr Sauerstoffverbrauch sehr gleichmässig ist.

In der vorliegenden Arbeit wird ferner über Versuche berichtet, in denen die Gewebsatmung von Meerschweinchen studiert wurde. Diese Versuchstiere wurden während des experimentellen Asthmas, das auf verschiedene Weise ausgelöst wurde, getötet oder starben in diesem Zustand. Das Ziel dieser Versuche war, aufzuklären, ob die im anaphylaktischen Schock nachgewiesene Herabsetzung der Gewebsatmung (ABDERHALDEN und WERTHEIMER, BÜNGELER), die eine so grosse Rolle für die Deutung der Veränderungen in der Sauerstoffaufnahme während des Schockes spielt, für diesen Zustand spezifisch ist, oder auch bei anderen Formen von Stenoseatmung vorkommt. Hierdurch sollte versucht werden, wenn möglich, eine grössere Klarheit über die Ursachen für die Herabsetzung der oxydativen Prozesse in den Geweben zu gewinnen.

Obgleich ich mir wohl bewusst bin, welch intime Beziehung zwischen Atmung und Kreislauf besteht, sehe ich in dieser Arbeit davon ab, näher auf die Kreislaufsprobleme einzugehen, und stelle dies für eine spätere Arbeit zurück. Ich glaube hierzu berechtigt zu sein, da sich aus den Versuchen, die hier besprochen werden sollen, sichere Schlüsse ziehen lassen, ohne auf die Reaktionen des Kreislaufes näher einzugehen. Die Veränderungen der Sauerstoffaufnahme bei schwerem experimentellen Asthma, die im folgenden ausführlich besprochen werden sollen, können nicht dadurch erklärt werden, dass sie eine Folge von primären Funktionsstörungen im Kreislaufsapparat wären.

*Zusammenfassend ist zu sagen: Ziel dieser Arbeit war es, zu einer einheitlicheren Auffassung über das Verhalten des Gaswechsels und der Lungenventilation während der Stenoseatmung zu gelangen. Zu diesem Zweck mussten der Gasaustausch und die Lungenventilation vor, während und nach einer Art von*

*Stenoseatmung mit möglichst einwandfreien Methoden studiert werden. Für diese Untersuchung schien das experimentelle Asthma des Meerschweinchens am geeignesten zu sein. Ferner war der Gewebsstoffwechsel bei dieser Form der Stenoseatmung zu untersuchen, um aufzuklären, ob sich die oxydativen Prozesse in den Geweben hierbei ebenso veränderten, wie nach älteren Erfahrungen im anaphylaktischen Schock.*

Derartige Untersuchungen über die Lungenventilation, den Gaswechsel und die oxydativen Prozesse in den Geweben wurden bei experimentellem Asthma bisher nicht durchgeführt. Bevor meine eigenen Versuche in dieser Richtung beschrieben werden, soll eine kurze Übersicht über die wichtigsten Untersuchungen gegeben werden, die zur Frage des experimentellen Asthmas in der Literatur vorliegen.

## KAP. II.

### Experimentelles Asthma.

Unter experimentellem Asthma wird in dieser Arbeit ein Zustand erschwerter Atmung verstanden, der durch Inhalation ausgelöst wird und vor allem durch eine exspiratorische Dyspnoe charakterisiert ist. Dieser Zustand kann anaphylaktisch oder auch nicht anaphylaktisch bedingt sein.

BUSSON (1911) zeigte als erster, dass man Meerschweinchen gegen eine bestimmte Art Eiweiss (Rinderserum) dadurch sensibilisieren kann, dass man das betreffende Eiweiss in Form eines feinen Nebels, der durch Spraybildung entsteht, einatmen lässt. Wurde einige Wochen später das Antigen injiziert, so zeigten die Versuchstiere alle charakteristischen Zeichen der Anaphylaxie (gesträubte Haare, Zittern, Seitenlage, Dyspnoe, Schüttelfrost, Abgang von Stuhl und Urin). Dagegen zeigte sich, dass es erheblich schwieriger war, an sensibilisierten Versuchstieren durch Einatmung des betreffenden Antigens in Form eines feinen Nebels anaphylaktische Symptome auszulösen. Einer Reihe von Verfassern gelang es nicht, auf diese Weise anaphylaktische Symptome zu erzeugen.

BUSSON und OGATA (1924) kamen zu dem Ergebnis, dass sensibilisierte Meerschweinchen häufig und während längerer Zeit dem Nebel exponiert werden mussten, wenn man durch Inhalation anaphylaktische Symptome hervorrufen wollte.

STORM VAN LEEUWEN, BIEN und VAREKAMP (1924) erzeugten an Meerschweinchen Dyspnoe, Jucken und Niesen dadurch, dass sie die Tiere wiederholt einige Zeit in einem Käfig mit milbenhaltigem Korn hielten. Die Symptome, die dabei auftraten, waren denen beim menschlichen Asthma bronchiale und bei der Rhinitis vasomotorica ähnlich. Dagegen gelang es nicht, auf die gewöhn-

liche Art und Weise durch die sensibilisierende intravenöse Injektion und die nachfolgende Schockdosis eines Extraktes von milbenhaltigem Korn anaphylaktische Symptome auszulösen. Es war den Verfassern auch nicht möglich, an Meerschweinchen, denen vorher Pollenextrakt oder Pferdeserum injiziert worden war, durch Inhalation des betreffenden Antigens anaphylaktische Symptome zu erzeugen.

ALEXANDER, BECKE und HOLMES (1926) fanden, dass man bei Meerschweinchen in der Mehrzahl der Fälle (25 von 31) durch Inhalation des Antigens dann anaphylaktische Symptome auslösen konnte, wenn die Versuchstiere kräftig sensibilisiert waren und ein starkes Antigen, z. B. Hühnereiweiss, angewendet wurde. Dass trotzdem der positive Effekt nicht 100 % -ig war, könnte man, nach der Ansicht dieser Autoren, dadurch erklären, dass der grösste Teil des Antigens in den Oesophagus und Magen, und nur ein geringer Teil in die Lungen kam. Dies konnte durch Inhalation einer Methylenblaulösung nachgewiesen werden. Diese Autoren sind der Meinung, dass die durch Inhalation erzeugten anaphylaktischen Symptome nicht mehr an Asthma erinnern, als die, die man bei Zufuhr des Antigens auf irgend eine andere Weise hervorrufen kann. Dale-Präparate von Tieren, die bei der Inhalation anaphylaktische Symptome bekamen, reagierten negativ.

MANTEUFEL und PREUNER (1933) untersuchten die Wirkung der Inhalation einer Lösung von Hühnereiweiss an Meerschweinchen, die gegen dieses Antigen sensibilisiert waren. Die Verfasser bestätigten die Resultate, zu denen BUSSON und OGATA, sowie ALEXANDER, BECKE und HOLMES gekommen waren. Die Verfasser betonen, dass die Teilchengrösse bei der Inhalation kleiner als  $5 \mu$  sein muss, wenn die Tröpfchen die feinsten Verästelungen des Bronchialbaumes und die Alveolen erreichen sollen. Die Autoren konnten ferner die wichtige Tatsache feststellen, dass die Meerschweinchen bei dieser Form der Anaphylaxie nach dem Anfall nicht anti-anaphylaktisch werden. Dies ist ein wichtiger Unterschied gegenüber dem klassischen anaphylaktischen Schock. Eine andere Differenz zeigte sich darin, dass die Symptome, die bei der Inhalations-Anaphylaxie im Vordergrund stehen, Atemnot und ausgesprochene exspiratorische Dyspnoe sind, während

im klassischen Anaphylaxie-Versuch allgemeine klonische Krämpfe das dominierende Symptom darstellen. In dieser Hinsicht ähnelt, nach MANTEUFEL und PREUNER, das experimentelle Asthma mehr dem Asthma bronchiale, als dem klassischen anaphylaktischen Schock. Ein Vorteil der Inhalationsmethode ist, dass sie es leicht möglich macht, die Stärke der anaphylaktischen Symptome zu variieren.

Wie KALLÓS und PAGEL (1937) zeigten, erhält man nach der Inhalation von Histamin und Acetylcholin das gleiche Symptomenbild (exspiratorische Dyspnoe, trockenen Husten und eine eigentümliche Körperhaltung, die dadurch gekennzeichnet ist, dass die Tiere auf den Hinterbeinen sitzen und den Kopf aufrecht und rückwärtsgeneigt halten), wie es nach der Inhalation von Eiweiss an Tieren auftritt, die vorher gegen dieses Antigen sensibilisiert waren. Der trockene Husten löst sich gegen Ende des Anfalles und die Tiere expectorieren Schleim. Dieser ist nach der Inhalation von Histamin und Acetylcholin zellarm, nach der Inhalation von Eiweiss (bei vorher sensibilisierten Tieren) dagegen enthält er reichlich eosinophile Leukocyten. Auch KALLÓS und PAGEL fanden nach diesen Anfällen keine Anti-Anaphylaxie. Der Dale-Versuch war nach diesen Autoren im Anschluss an den Anfall positiv. Dies stimmt nicht mit den Befunden von ALEXANDER, BECKE und HOLMES überein. Das Fehlen der Anti-Anaphylaxie soll nach Coca u. a. für die Zustandsbilder der Idiosynkrasie beim Menschen charakteristisch sein, im Gegensatz zu dem Verhalten bei experimenteller Anaphylaxie. KALLÓS und PAGEL betonen, dass mit dem Nachweis des Fehlens der Anti-Anaphylaxie beim experimentellen Asthma diese Auffassung definitiv widerlegt ist. Von grossem Interesse ist der Unterschied im histologischen Bild der Lungen, den diese Verfasser einerseits bei den Versuchstieren nachwiesen, die Histamin oder Acetylcholin eingeatmet hatten, und andererseits bei denen, die nach vorheriger Sensibilisierung das homologe Antigen inhalierten. Während die erstere Gruppe ein uncharakteristisches Bild chronischer Bronchitis zeigt, wobei die Gefässveränderungen dominieren, findet man bei den allergischen Tieren eine äusserst starke Eosinophilie in der Submucosa der mittelgrossen und kleinen Bronchien. Die Bronchiallumina sind

teilweise mit Thromben gefüllt, die eosinophile Leukocyten enthalten. KALLÓS und PAGEL sind der Meinung, dass die Einatmung des homologen Antigenes bei sensibilisierten Meerschweinchen ein Krankheitsbild erzeugt, das klinisch, pathologisch-anatomisch, histologisch und immunbiologisch mit dem Asthma bronchiale des Menschen identisch ist.

Wie KALLÓS und KALLÓS-DEFFNER (1937) durch Röntgenaufnahmen mit Lipiodol-Kontrast zeigten, entsteht beim experimentellen Asthma eine Verengerung der mittelgrossen Bronchien. Die Röntgenbilder zeigten dabei ausserdem ein hochgradiges Emphysem.

EWERT und KALLÓS (1937) untersuchten das Elektrokardiogramm von Meerschweinchen während eines experimentellen Asthmas, das durch Inhalation des homologen Antigenes an sensibilisierten Tieren oder durch Einatmung von Histamin bzw. Acetylcholin an normalen Tieren ausgelöst wurde. Während des Asthmas traten, gleichgültig wie es hervorgerufen wurde, elektrokardiographische Veränderungen auf. Sie beruhten nach der Deutung der Verfasser auf einer Anoxämie. Nach dem Abklingen des Asthmaanfalles wurde das Elektrokardiogramm wieder normal.

RATNER (1939) erzeugte durch Inhalation von »dry horse dander« experimentelles Asthma an Meerschweinchen, die vorher das gleiche Antigen eingeatmet hatten. Die Tiere bekamen in der Regel deutliche anaphylaktische Symptome, wenn sie genügend lange sensibilisiert waren. Sie zeigten meist keine Anti-Anaphylaxie. Der Dale-Versuch war in manchen Fällen positiv, in anderen negativ. Der Verfasser schliesst hieraus, dass in manchen Fällen nur eine lokale Sensibilisierung der Respirationsorgane stattfand, während bei den übrigen Fällen auch andere Organe sensibilisiert wurden.

In einer späteren Arbeit untersuchten KALLÓS und KALLÓS-DEFFNER (1942) an Meerschweinchen die Wirkung von Forssman-Antiserum. Auch mit diesem bekommt man ein Symptomen-Bild, das im Grossen und Ganzen den anderen Formen des experimentellen Asthmas gleicht. Es unterscheidet sich von diesen nur dadurch, dass die Atmung zwar erschwert ist, aber der exspiratorische Typus der Dyspnoe fehlt. Bei der Röntgenuntersuchung

der Lungen unter Kontrastfüllung der Luftröhre findet man hier auch keine Verengerung des Bronchiallumens. Bei schwereren Symptomen sterben die Versuchstiere unter asphyktischen Krämpfen. Im histologischen Bild dominieren Gefässveränderungen, vor allem an den Arteriolen, die oft von perivasculären Blutungen umgeben sind. Das Fehlen der Eosinophilie ist auch für das auf diese Weise erzeugte experimentelle Asthma charakteristisch.

Über die Ursachen des experimentellen Asthmas schreiben KALLÓS und KALLÓS-DEFFNER (1942) folgendes: »Die Asthmaanfälle, die bei Meerschweinchen durch Histamin- bzw. Acetylcholineinatmung ausgelöst werden, sind als rein (oder überwiegend) bronchospastisch bedingt aufzufassen. Die Anfälle, die nach Einatmung des Forssman-Antiserum bei Meerschweinchen auftreten, beruhen nach der vorliegenden Untersuchung in der Hauptsache auf einer Gefässschädigung in der Lunge, zu der Veränderungen des Parenchyms hinzukommen. Die Asthmaanfälle schliesslich, welche bei allergischen Meerschweinchen durch Einatmung des homologen Allergens ausgelöst werden können, lassen eine Beteiligung aller dieser Komponenten erkennen».

Zusammenfassend ist zu sagen, dass das experimentelle Asthma zwar Gegenstand eingehender pathologisch-anatomischer, histologischer, serologischer und immunbiologischer Studien war, dass aber — abgesehen von der Arbeit von KALLÓS und EWERT — keine eingehenderen pathophysiologischen Untersuchungen hierüber vorliegen.

## KAP. III.

### Methodik.

#### 1. Geschlossenes Respirationssystem.

a.) *Kontinuierliche Registrierung des Sauerstoffverbrauches bei normalem oder erhöhtem Sauerstoffgehalt.*

Für diese Versuchsanordnung wendete ich im Prinzip die Methode an, die von KROGH und LINDBERG (1931 und 1932) für die Bestimmung des respiratorischen Gaswechsels bei kleinen Versuchstieren ausgearbeitet wurde. Gewisse Modifikationen in der Apparatur waren notwendig, um aus der Lösung, die das Asthma hervorrufen sollte, einen Nebel bilden zu können. Diese Veränderungen bestanden in der Einschaltung von zwei Spray-Apparaten und einer abgeänderten Konstruktion der Pumpe. Die Abbildungen 1 und 2 zeigen das Aussehen der Apparatur. Abb. 2 gibt ein vergrössertes Detailbild von Abb. 1. Ich prüfte verschiedene Typen von Pumpen, von denen sich die in Abb. 1 verwendete als die Beste erwies. Die Pumpe, die in der Abb. mit 1 bezeichnet ist, hatte die Nebelspray-Bildung und die Luftzirkulation im System zu bewirken. Wie aus der Abb. hervorgeht, besteht die Pumpe aus zwei mit Ventilen versehenen Gummiballons, von denen jeder ein Volumen von 450 cc hat. Diese Ballons werden abwechselnd von aussen zusammengepresst. Hierdurch entsteht ein diskontinuierlicher Luftstrom, der eine wirkliche Nebelbildung möglich macht. In den meisten Versuchen betrug das angewendete Zirkulationsvolumen 4 Liter pro Minute. Die Luft ging zuerst durch den Schlauch 2, dann durch eine spiralenförmige Zinnröhre, die sich im Wasserbad 3 befindet, und schliesslich zu den Spray-Apparaten 4 und 5. Von diesen

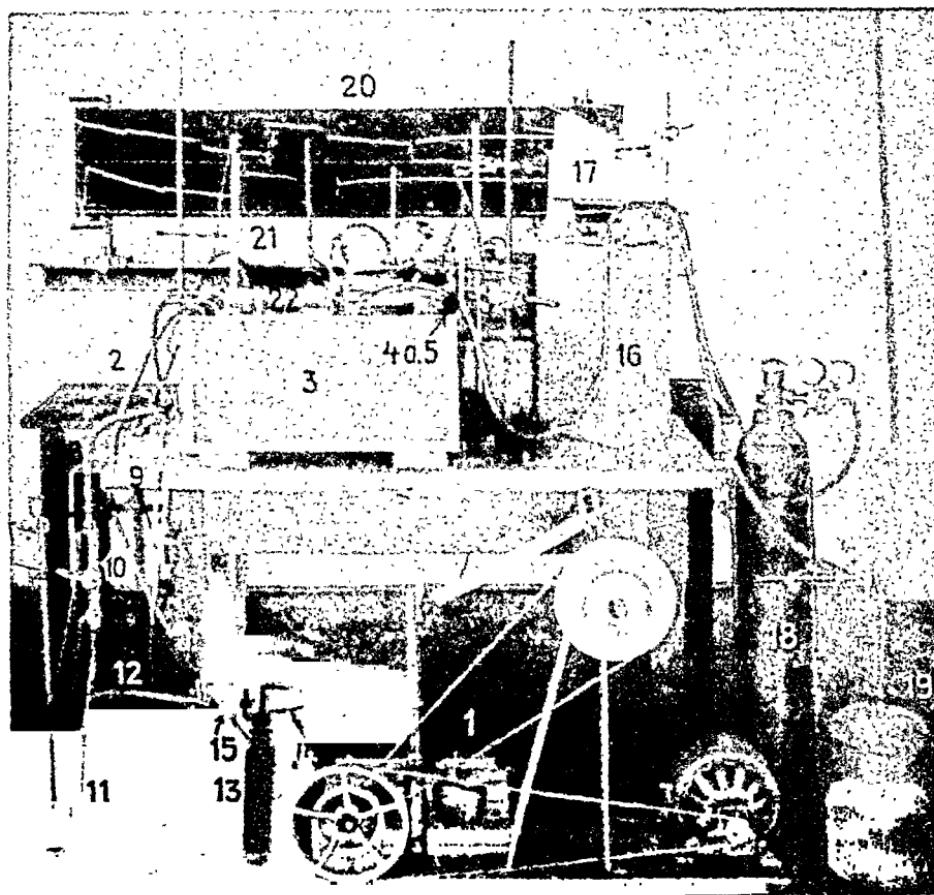


Abb. 1. Geschlossener Respirations-Apparat.  
Erklärung der Zahlen-Bezeichnungen im Text.

enthält der eine destilliertes Wasser — er wird in der Vor- und Nachperiode eingeschaltet — der andere die Lösung, durch die das Asthma ausgelöst wird, — dieser wird während der Asthma-periode eingeschaltet —. Durch einfaches Umsetzen einer Schlauchklemme schaltet man von dem einen auf den anderen Spray-Apparat um. Dabei entsteht auf der Kurve ein kleiner Knick, der auf Abb. 3 deutlich zu erkennen ist. Dieser Knick ist durch die Senkung des Druckes im System bedingt, die entsteht, wenn beide Spray-Apparate eingeschaltet sind. Von den Spray-Apparaten gelangt die Luft in die Versuchstier-Kammer 6. Im Wasserbad gibt es eine weitere Tierkammer 7. Diese wird aber nicht während des Versuches selbst benutzt. In ihr sollen sich die Versuchstiere an die Versuchsbedingungen gewöhnen.

Der Boden der Tierkammer ist mit Holz, ihre Wände sind mit wärmeisolierendem Material verkleidet. Die Einmündungen der zu- und abführenden Leitungen in der Tierkammer sind mit Metallnetzen bedeckt, um ihre Verstopfung zu verhindern, falls sich das Versuchstier gegen sie drücken würde. Von der Tierkammer geht die Luft durch den Schlauch 8. An diesem befindet sich, durch einen Gummischlauch eingeschaltet, ein Zwei-Wege-Hahn 9, der es möglich macht, während des Versuches dem Apparat Luftproben zu entnehmen. Der Schlauch 8 ist an einen Wasserkühler 10 angeschlossen, um die übersättigte Feuchtigkeit zu kondensieren. Diese wird aus der Apparatur durch ein wassergefülltes U-Rohr 11 entfernt. Durch den Wasserabschluss in diesem ist der Apparat gegen die Aussenluft geschlossen. Nach der Abkühlung wird die Luft durch den Schlauch 12 in die Kohlensäure-Absorptions-Flasche 13 geleitet, die Natronkalk enthält. Von hier gelangt sie schliesslich durch den Schlauch 14 zurück zur Pumpe. Zwischen den Schläuchen 12 und 14 besteht ausserdem eine direkte Verbindung durch Schlauch 15, wodurch die Kohlensäure-Absorptions-Flasche umgangen werden kann. Diese Verbindung ist bei der Bestimmung des Sauerstoffverbrauches des Versuchstieres durch eine Schlauchklemme verschlossen. Durch den Schlauch 16 steht das Spirometer 17 mit der Tierkammer in Verbindung. Das Spirometer kann entweder mit Sauerstoff aus der Sauerstoffbombe 18, oder mit Luft aus der Glasflasche 19 gefüllt werden, in der die Luft über Wasser von Zimmertemperatur steht. Die Bewegungen der Spirometer-Glocke wurden auf dem Kymographion 20 registriert. Die Temperatur im Wasserbad 3 wurde durch eine Thermo-Relais-Anlage, deren Kontaktthermometer 21 und Tauchsieder 22 auf der Abbildung zu sehen ist, auf  $0,1^{\circ}$  genau konstant gehalten. Durch den Propeller 23 wird das Wasser im Wasserbad umgerührt. Das Dach des Tierkastens, in das ein Glasfenster eingesetzt ist, hat eine umgebogene Kante, die unter den Wasserspiegel reicht. Hierdurch wird das System luftdicht geschlossen, nachdem das Tier hineingesetzt wurde. Durch Röhren im Deckel gehen die Leitungen 24 zu den Thermoelementen, durch die die Rektal- und Hauttemperatur des Versuchstieres gemessen werden. Die eine Lötstelle des Thermoelementes befindet sich auf dem Versuchs-

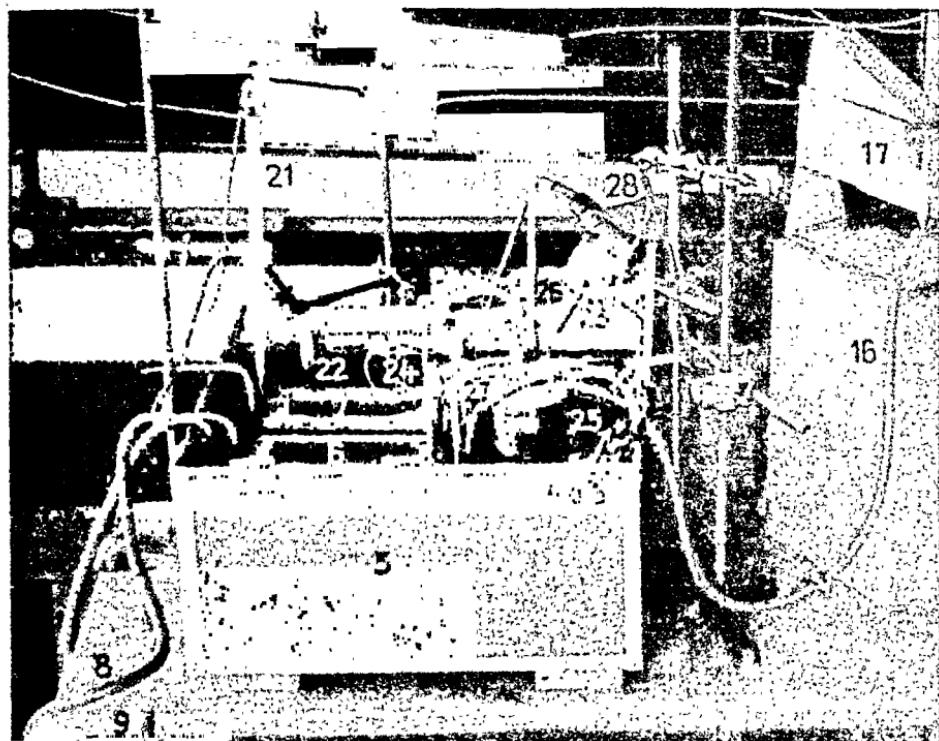


Abb. 2. Detailbild von Abb. 1.

tier, die andere in einer Thermosflasche 25, deren Temperatur auf dem Normalthermometer 26 abgelesen wird. Die verschiedenen Thermoelemente können der Reihe nach durch den Umschalt-Schlüssel 27 und den Leitungsdräht 28 mit dem Galvanometer in Verbindung gesetzt werden. Der Gesamtrauminhalt des Systems war ca. 3000 cc. Dieser verminderte sich um das Volumen des Versuchstieres, nachdem dieses hineingesetzt worden war. Die ganze Apparatur war in einem Thermostat-Raum aufgestellt, in dem die Temperatur konstant auf 21 Grad gehalten wurde.

Die Temperatur des Wasserbades wurde auf 30 Grad gehalten, da KROGH und LINDBERG (1931) zeigten, dass bei höherer Temperatur, bis zu einer gewissen Grenze, die Unterschiede zwischen den verschiedenen Bestimmungen des Sauerstoffverbrauches am gleichen Versuchstier geringer sind. Diese Autoren stellten auch fest, dass eine Temperatur von  $30^{\circ}$  im Wasserbad für derartige Versuch am geeignetsten ist. Der Sauerstoffverbrauch der Versuchstiere ist bei dieser Temperatur niedriger als bei Zim-

mertemperatur. Dies beruht auf dem Fehlen des leichten Muskel-tremors, den die Tiere bei niedrigerer Temperatur haben. Wenn die Temperatur des Wasserbades 30 Grad, ist, ist die Luft-temperatur in der Tierkammer 1 bis 2 Grad höher, aber bei gleichmässiger Zirkulationsgeschwindigkeit im System konstant.

Die Luft im System wird bald nach Beginn des Versuches durch die Spray-Apparate wie durch die Ausatmung des Ver-suchstieres mit Feuchtigkeit gesättigt. HEMMINGSEN (1933) zeigte, dass die Luft in einem Respirations-Apparat ähnlicher Konstruk-tion schon durch den Wasserdampf, den das Versuchstier ausat-met, praktisch genommen mit Feuchtigkeit gesättigt wird.

Die Zirkulationsgeschwindigkeit der Luft im System wurde wäh-ren der Versuche stets konstant gehalten. Sie betrug meist ungefähr 4 Liter pro Minute. In den Versuchen, in denen der Sauerstoffverbrauch bestimmt wurde, schwankte das Zirkula-tionsvolumen zwischen 2,5 und 5 Litern pro Minute. Der Kohlen-säure-Gehalt im System hielt sich, wie Gasanalysen zeigten, un-ter diesen Bedingungen bei ungefähr 0,30 % mit nur geringen Abweichungen. Dieser Kohlensäure-Gehalt ist nach KROGH (1916, Seite 36) für die Tiere irrelevant.

Die Spray-Apparate waren ganz aus Glas (Fabrikat Weil). Diejenigen sind am besten, deren Kapillaren einen möglichst kleinen inneren Durchmesser haben. Aus einer Anzahl von Spray-Apparaten wurden zwei ausgewählt, deren Kapillaren genau gleich waren. Um dies beurteilen zu können, wurde an den verschiedenen Spray-Apparaten die Geschwindigkeit eines Luftvolumens bestimmt, das unter einem bestimmten konstanten Druck hindurchgetrieben wurde. Die Flüssigkeitsmenge, die bei der gewöhnlichen Durchströmungsgeschwindigkeit der Luft im System in einer Stunde verbraucht wurde, betrug ungefähr 1 cc. Ich hielt es nicht für nötig, die Tropfengrösse in dem gebildeten Nebel zu bestimmen, da die angewandten Spray-Apparate ein ausgezeichnetes Asthma hervorriefen.

Obgleich der Druck in den Gummiballons und in den Leitungen zu den Spray-Apparaten recht erheblich war, hatte ich keine grösseren Schwierigkeiten, den Apparat dicht zu halten. Es ist dies allerdings nur möglich, wenn der Gang der Ventile unter Benutzung eines ziemlich festen Kranfettes gut geschmiert ist. Ich wen-

dete ein Kranfett von folgender Zusammensetzung an, wie es auch für Gasanalysen-Apparate benutzt wird: 9 gr. Rohgummi, 8 gr. Paraffin und 32 gr. Vaseline. Der Rohgummi wurde fein zerschnitten und die Mischung auf dem Wasserbad vorsichtig erwärmt, bis sich eine homogene Lösung gebildet hatte. Nach Beendigung eines Versuches wurde die Apparatur stets auf Dichte geprüft, indem das System ohne Versuchstier in Gang gesetzt wurde. Die Spirometer-Glocke soll in diesem Fall auf dem Kymographion eine gerade Linie schreiben. Häufig wurde die Kontrolle dadurch verschärft, dass im System Über- oder Unterdruck hervorgerufen wurde, ersterer durch Belastung der Spirometerglocke, letzterer durch Erhöhung des Gegengewichtes. Es dauert etwa 20 Minuten nach dem Ingangsetzen des Apparates, bis die Linie gerade wird, auch wenn die Apparatur dicht ist. Dies hat seinen Grund darin, dass eine gewisse Zeit vergeht, bis die Temperatur- und Feuchtigkeitsverhältnisse im System konstant werden. Dies Verhalten wird durch die Kurve in Abbildung 1 verdeutlicht, auf der die von dem Spirometer registrierte Linie in der ersten Zeit, nachdem die Apparatur in Gang gesetzt wurde, bogenförmig ansteigt. Wenn aber im Verlaufe eines Versuches eine kürzere Unterbrechung von einigen Minuten Dauer stattfindet, werden die Verhältnisse fast sofort konstant. Dies kann wahrscheinlich dadurch erklärt werden, dass in diesem Fall die Temperatur viel racher konstant wird, da die verschiedenen Teile der Apparatur während dieser kurzen Zeit ihre Temperatur nicht wesentlich verändern. Die neue Luft, die jetzt eventuell dem System zugeführt wurde, nimmt sehr rasch die Umgebungs-temperatur an.

Wenn sich ein Versuchstier im Apparat befindet und dieser dicht ist, so registriert das Spirometer, nach Eintreten von Temperatur- und Feuchtigkeits-Konstanz, eine gerade abfallende Linie, falls der Sauerstoffverbrauch des Tieres konstant ist und sich ausserdem der Kohlensäure-Gehalt des Systemes nicht ändert. Wie aber bereits oben betont, hält sich der Kohlensäure-Gehalt im System praktisch genommen konstant. Das Aussehen einer derartigen Kurve geht aus Abbildung 3 hervor. Das vom Spirometer registrierte Volumen gilt für die Temperatur des Spirometers, bei Sättigung der Luft mit Feuchtigkeit und unter

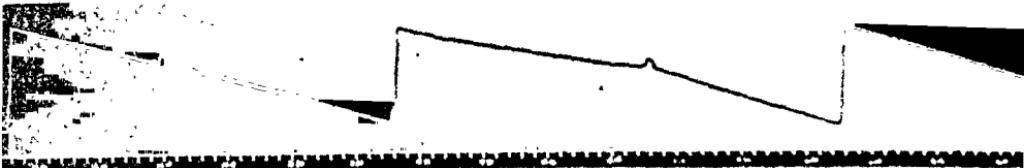


Abb. 3. Kurve aus dem Versuch vom 28.3.1940 (siehe auch Abb. 9). Sie zeigt den Sauerstoffverbrauch eines Meerschweinchens kurz vor, während und kurz nach einem experimentellen Asthma. Die Knieke in der Kurve geben jeweils den Zeitpunkt für die Umschaltung der Spray-Apparate an. Zeitmarkierung in Minuten.

dem jeweils herrschenden Barometerdruck. Das abgelesene Volumen muss dann auf 0 Grad, 760 mm Druck und Trockenheit reduziert werden.

In manchen Versuchen wurde ein höherer Sauerstoff-Gehalt, als der für Luft Normale, im System hergestellt, da sich zeigte, dass die Versuchstiere unter diesen Bedingungen längere Zeit ein schwereres Asthma aushalten konnten. In verschiedenen Versuchen wurde der Sauerstoffgehalt zwischen 50 und 90 % variiert. Durch Analysen von Gasproben, die dem System entnommen wurden, wurde stets die genaue Zusammensetzung der Atmosphäre in der Apparatur bestimmt. Während der Entnahme dieser Gasproben wurde der Schlauch von der Tierkammer zu dem Spirometer abgesperrt. Wenn die Verbindung zum Spirometer wieder geöffnet wurde, fiel die Spirometerglocke um ein Stück, das dem entnommenen Gasvolumen entsprach. Wie sich dies auf der Kurve darstellt, geht aus der in Abbildung 1 wiedergegebenen Kurve hervor. Die Vorperiode war stets lange genug, damit sich das Versuchstier mit der im System vorhandenen Zusammensetzung der Atmosphäre ins Gleichgewicht setzen konnte. Andererseits wurden die Versuche niemals so lange ausgedehnt, dass es zu einer schädlichen Wirkung des erhöhten Sauerstoff-Gehaltes hätte kommen können. Dies ist nach den übereinstimmenden Angaben der Literatur erst nach mehreren Tagen der Fall. Irgend ein derartiger Effekt konnte auch nicht festgestellt werden.

### Die Genauigkeit der Methode.

Nachdem in den oben besprochenen Verhältnissen Konstanz eingetreten war, beruhte die Genauigkeit der Methode allein auf dem Grad von Exaktheit, mit dem das Sinken des Spirometers abgelesen werden konnte. Das Volumen des Spirometers war 350 cc. 50 cc entsprechen einem Fallen der Kurve um 16 mm. Die Meerschweinchen, die für die Respirationsversuche benutzt wurden, wogen ungefähr 600 gr. Ihr Sauerstoffverbrauch betrug im allgemeinen etwa 8 cc pro Minute. Wurde der Sauerstoffverbrauch in 5-Minuten-Perioden bestimmt, so musste die Kurve um ca. 13 mm fallen. Man kann ohne Schwierigkeit ein Sinken der Kurve um 0,3 mm genau ablesen. Alle Ablesungen wurden kontrolliert. Der Fehler der Methode beträgt daher bei Ablesung während 5-Minuten-Perioden und unter den oben erwähnten Bedingungen weniger als 2,5 %. Bei höherem Sauerstoffverbrauch und längeren Ablesungsperioden wird das Fehlerprozent kleiner, bei niedrigerem Sauerstoffverbrauch grösser. Kürzere Ablesungsperioden als 5 Minuten kamen nur in Ausnahmefällen vor.

### b.) Methode zur Erzeugung einer niedrigen Sauerstoff- und einer hohen Kohlensäure-Spannung im geschlossenen Respirationsystem.

Aus besonderen Gründen untersuchte ich an Meerschweinchen die Effekte einer niedrigen Sauerstoff-Spannung bei normalem Kohlensäure-Gehalt, die einer erhöhten Kohlensäure-Spannung bei normalen Sauerstoff-Gehalt und schliesslich die einer gleichzeitigen Einwirkung von niedriger Sauerstoff-Spannung und erhöhtem Kohlensäure-Gehalt. Für alle diese verschiedenen Versuchsbedingungen eignet sich das oben beschriebene geschlossene Respirationsystem ausgezeichnet.

*Niedrige Sauerstoffspannung* wurde dadurch bewirkt, dass die Glocke des Krogh'schen Spirometers statt mit Sauerstoff mit Luft gefüllt wurde. Hierdurch nimmt der Sauerstoff-Gehalt im System allmählich und gleichmässig ab. Dies ist von Bedeutung, wenn man will, dass die Versuchstiere die verminderte

Sauerstoffspannung möglichst lange aushalten sollen. Dadurch, dass man die Krogh-Glocke mit einem bestimmten Mengenteil Luft und einem bestimmten Teil Sauerstoff füllt, kann man nach Wunsch die Geschwindigkeit, mit der die Sauerstoffspannung im System abnimmt, bestimmen. Füllt man die Glocke mit 300 cc Luft, so bedeutet dies, dass der Sauerstoff-Gehalt des Systems nach Entleerung der Glocke um ca. 50 % gesunken ist. Das Versuchstier atmet nämlich in der betreffenden Zeit 300 cc Sauerstoff ein. Diese werden dem System entnommen. In der gleichen Zeit werden aber nur ca. 60 cc Sauerstoff dem System zugeführt. Der Sauerstoff-Gehalt in ihm vermindert sich daher um etwa 240 cc. Da das System ursprünglich ca. 500 cc enthielt, hat sich sein Sauerstoff-Gehalt um 50 % vermindert. Der Sauerstoffverbrauch des Versuchstieres wurde so bestimmt, wie es unter a beschrieben wurde.

Auch wenn man die Spirometerglocke mit Sauerstoff füllt, sinkt der Sauerstoffgehalt im System. Dies beruht auf der Beimengung von einigen Prozent Stickstoff zum Sauerstoff. Rechnet man damit, dass der Sauerstoff 1 % Stickstoff beigemengt enthält, so werden mit jeder Entleerung der Glocke dem System 3 cc Stickstoff zugeführt. Bei dem Sauerstoffverbrauch, den die Tiere hatten, bedeutet dies die Zufuhr von ca. 6 cc Stickstoff in der Stunde. Bei einem Versuch von 10-stündiger Dauer macht dies ca. 60 cc. Infolgedessen vermindert sich der Sauerstoffgehalt im System um 12 %. Dies hat aber keinerlei Bedeutung. Bei manchen langdauernden Versuchen wurde trotzdem eine Lüftung des Systems vorgenommen, um die Versuchsbedingungen möglichst konstant zu halten.

*Der erhöhte Kohlensäure-Gehalt im System kam durch Herabsetzung der Zirkulationsgeschwindigkeit auf etwa 0,15 bis 0,50 Liter pro Minute zu Stande. In manchen Versuchen wurde ausserdem nur ein Teil der Luft im System durch die Kohlensäure-Absorptions-Flasche geleitet. Dies geschah dadurch, dass ganz oder teilweise der Nebenschluss-schlauch 15 in Abbildung 1 geöffnet wurde. Auf diese Weise kann man verschiedene Kohlensäure-Spannungen im System zwischen 1 und 5 % erhalten. Nach ungefähr  $\frac{1}{2}$  Stunde wird bei unveränderten Versuchsbedingungen der Kohlensäure-Gehalt im System ziemlich*

konstant, wobei er aber doch zwischen 0,5 und 1 % schwanken kann. Der Sauerstoffverbrauch des Versuchstieres kann daher nicht mit einigermassen grosser Genauigkeit berechnet werden. Eine derartige Berechnung wurde deshalb auch nicht durchgeführt.

In manchen Versuchen wurde die Einwirkung des erhöhten Kohlensäure-Gehaltes auf das Versuchstier so untersucht, dass das geschlossene System in ein offenes verändert wurde. In diesem Fall wurde Schlauch 14 an einen Douglassack angegeschlossen, der fortlaufend aus einer Gasbombe mit einer Gasmischung mit bekanntem Kohlensäure-Gehalt gefüllt wurde.

*Die gleichzeitige Einwirkung einer niedrigen Sauerstoffspannung und einer hohen Kohlensäurespannung* wurde durch eine Kombination der oben angegebenen Verfahren hervorgerufen. Die Glocke des Krogh'schen Spirometers wurde mit Luft gefüllt und gleichzeitig die Zirkulationsgeschwindigkeit im System vermindert. Bestimmungen des Sauerstoffverbrauchs wurden in diesen Versuchen aus den oben angegebenen Gründen nicht ausgeführt. Durch Gasanalysen wurde die genaue Gaszusammensetzung im System wiederholt im Verlaufe der Versuche bestimmt.

## 2. Offenes Respirationssystem nach der Methode von Douglas.

### a.) *Nicht narkotisierte Versuchstiere.*

Die Versuchsanordnung geht aus Abbildung 4 hervor. Die Aussenluft, die durch ein Ventil in der Aussenwand eintritt, wird von der gleichen Pumpe, die für das geschlossene Respirationssystem angewendet wurde, durch den Schlauch 1 weiter gepumpt und durch die Sprayapparate 2 gedrückt. Auch diese sind von gleicher Art, wie weiter oben beschrieben. Von den Spray-Apparaten wird die Luft in eine offene tubulierte Flasche 3 geleitet, deren Rauminhalt etwa 1000 cc ist. Durch ihren offenen Hals ist ein Schlauch eingeführt. Die Weite des Halses ist im Verhältnis zum Durchmesser des Schlauches Nr. 4 gross. Infolgedessen besteht keine Gefahr, dass die Luft in den Schlauch gedrückt

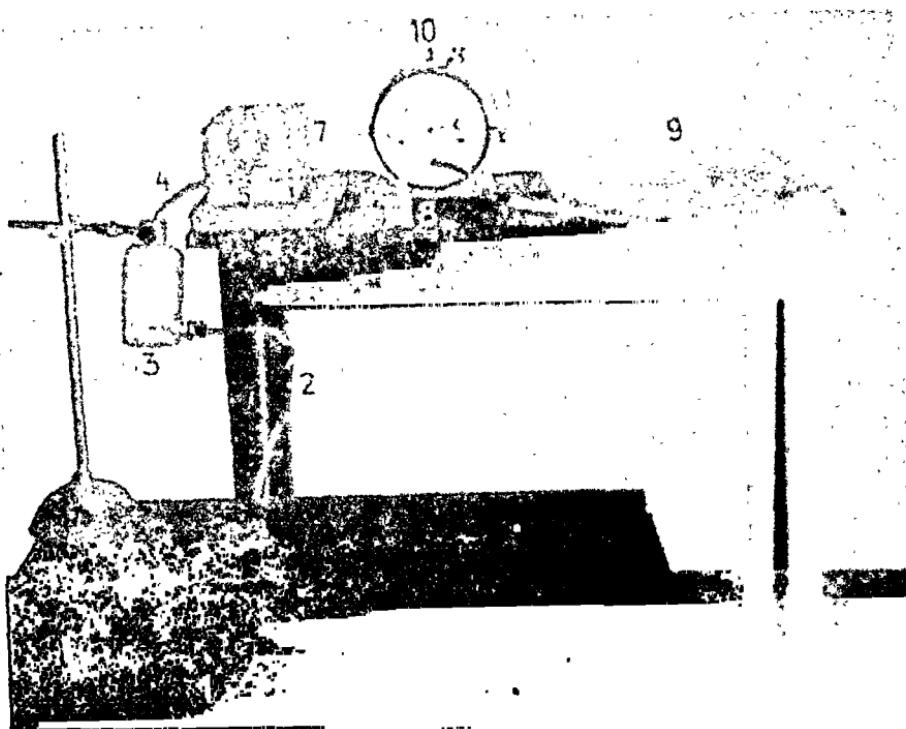


Abb. 4. Anordnung für die offene Respirationsmethodik nach Douglas.  
Erklärung der Hinweise im Text.

wird. Kontrollversuche zeigten, dass dies tatsächlich nicht geschieht. Der Schlauch 4 führt zum Atemventil 5, dieses steht in Verbindung mit der Atemmaske 6. Die beiden letzteren werden weiter unten näher beschrieben. Die Atemmaske 6 ist in einem Holzkasten 7 befestigt, in dem das Tier während des Versuches sitzt. Die Ausatmungsluft geht vom Ventil zu der Wasserkondensations-Flasche 8. Sie wird während 5- oder 10-Minutenperioden im Sack 9 gesammelt. Die in einer 5- oder 10-Minutenperiode gesammelte Luft wird im Sack durcheinandergemischt. Danach wird eine Gasprobe von 50 cc zur Analyse entnommen. Der Inhalt des Sackes wird dann mit möglichst gleichmässiger Geschwindigkeit durch die Gasuhr getrieben. Zu dem gefundenen Volumen werden 50 cc, entsprechend der entnommenen Gasprobe, addiert.

*Die Atemmaske:* Ihr Aussehen und ihre Konstruktion geht aus Abbildung 5 hervor. Sie ist aus Kautschuk von der Art,



Abb. 5. Atemmaske (Grösse 1:1).

wie er in der zahnärztlichen Praxis verwendet wird, angefertigt, direkt nach dem Abdruck eines Meerschweinchen-Kopfes. Ich verwendete zwei Masken von verschiedener Grösse, die eine passte für Meerschweinchen von 500 gr Gewicht, die andere für solche von 700 gr Gewicht. Im Vorderteil der Maske ist eine Öffnung, die inwendig mit einem Falz versehen ist. In dieser Öffnung ist durch eine Verschraubung eine Messingröhre befestigt. Durch eine Gummipackung wird die Verbindung luftdicht. Die Messingröhre ist durch eine Öffnung in einem Metallband hindurchgesteckt. In diesem ist sie durch die oben erwähnte Verschraubung befestigt. Das Metallband seinerseits ist an dem Kasten befestigt. Bevor die Maske dem Tier aufgesetzt wird, werden die Haare am Kopf und Oberkörper kurz geschnitten. Kopf und Hals werden mit einer dicken Schicht Vaseline eingeschmiert. Ein breiter Heftpflasterstreifen wird quer über dem Rücken des Versuchstieres, ein Stück unterhalb des Nackens, befestigt. Ein anderer Heftpflasterstreifen wird über die Brust des Tieres geklebt. Die Atemmaske wird durch Schnüre, die an ihr in Löchern befestigt sind, an den Heftpflasterstreifen festgebunden. Über die Atemmaske wird dann ein sehr dünner

Gummisack gezogen, der sich dem mit Vaseline eingeschmierten Hals eng anschliesst. Schliesslich wird die Atemmaske in dem Metallband befestigt und das Versuchstier in den Kasten gesetzt. Dadurch dass man die Öffnung der Messingröhre einen Augenblick verstopft, kann man leicht kontrollieren, ob der Gummisack dicht hält.

*Das Atemventil:* Es wurde ein Ventil vom Lovén-Typus benutzt. Seine nähere Konstruktion geht aus der Abbildung 6 hervor. Es besteht aus Glas, Gummikorken und zwei mit Flanschen versehenen Messingröhren. Die Klappen sind sehr dünne

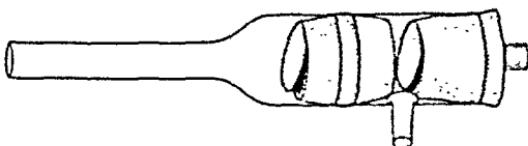


Abb. 6. Atemventil (Grösse 1:2).

Gummimembranen, die an den Metallflanschen der Messingröhre befestigt sind. Sie sind so leicht beweglich, dass sie keinen nennenswerten Widerstand verursachen. Der schädliche Raum des Ventils ist ungefähr 1 cc. Durch Kontrollversuche zeigte sich, dass die Ventile bei den Frequenzen und dem Atemvolumen, die bei Versuchen an Meerschweinchen in Frage kommen, untadelig funktionieren. In diesen Kontrollversuchen wurde eine Rekordspritze als Lunge benutzt.

*Der Sack:* Der Sack war aus einem papierdünnen Gummi, der Pliofilm heisst. Das Material war ein Fabrikat von Goodyear, und wurde mir von der Firma Åkerlund und Rausing, Lund, bereitwilligst zur Verfügung gestellt. Den Sack fertigte ich selbst an, indem ich ihn zusammen vulkanisierte. Dies geschah dadurch, dass eine mässig erwärmte Rolle über das Material geführt wurde. In der einen Ecke wurde ein gewöhnlicher Gummischlauch einz vulkanisiert. Selbst wenn man den Sack zweischichtig herstellt, ist das Material so leicht, dass ein derartiger Sack bei mässiger Füllung an einem Wassermanometer, mit dem man ihn verbunden hat, keinen Überdruck zeigt. Bläst man einen

derartigen Sack auf, so dauert es mehrere Stunden, bis er sich entleert, wenn man den Gummischlauch offen lässt, obgleich dieser einen grossen Durchmesser hat. Dieses Material ist, wie sich zeigte, gut für Säcke zur Aufnahme der Ausatmungsluft geeignet, da es für Sauerstoff und Kohlensäure schwer durchgängig ist. Dies geht aus den Analysen-Werten der Tabelle 1 hervor.

*Tabelle 1.*

Zeit nach der Füllung des Sackes.	Sauerstoff in %	Kohlensäure in %
0 Min.	62,59	11,11
15 Min.	—	11,42
30 Min.	62,51	11,37
1 Std. 50 Min.	—	11,33
3 Std. 30 Min.	62,57	—
ca. 24 Std.	62,07	—

In diesen Versuchen wurde für die Prüfung der Durchlässigkeit für Sauerstoff eine Gasmischung angewendet, die ungefähr 62,5 % Sauerstoff und 37,5 % Stickstoff enthielt, und für die Untersuchung der Durchlässigkeit für Kohlensäure eine Mischung aus ca. 11,5 % Kohlensäure, 18,5 % Sauerstoff und 70 % Stickstoff. In den gewöhnlichen Atemversuchen beträgt die Differenz zwischen der Ausatmungsluft und der Luft des Zimmers nur ca. 2 %. Da bei den Atemversuchen die Gasprobe zur Analyse unmittelbar nach einer 5- oder 10-Minuten-Periode entnommen wird, kann nach diesen Analysen-Werten keinerlei Veränderung der Ausatmungsluft in der Zeit, die für ihre Entnahme gebraucht wird, zu Stande kommen. Der Sack wurde unmittelbar darnach entleert, indem sein Inhalt durch die Gasuhr getrieben wurde.

*Die Gasuhr:* Eine Kontrollgasuhr mit Wasserfüllung von dem Typ, der in Abbildung 4 wiedergegeben ist, wurde angewendet. Eine ganze Umdrehung entsprach 1 Liter. Der Inhalt des Sackes wurde mit möglichst konstantem Druck durch die Gasuhr getrieben. Bei allen Messungen wurden grössere Mengen als 1 Liter

gebraucht. Auf diese Weise wird der mittlere Fehler in dem gemessenen Volumen kleiner als 0,5 %. Das bestimmte Volumen wurde auf 0 Grad, 760 mm und Trockenheit reduziert.

*Die Gasanalysen:* Diese wurden meist mit einem von KROGH modifizierten Haldane-Apparat ausgeführt, der mit einer genau ausgewogenen Bürette versehen war. Als mittlerer Fehler der einzelnen Bestimmungen wird im allgemeinen für diesen Apparat  $\pm 0,02$  (absolute Zahl) angegeben. Der Sauerstoffgehalt der Inspirationsluft ( $O_2$ ) musste nach folgender Formel korrigiert werden:

$$O_2 \text{ Korrig.} = O_2 \frac{N_{ex}}{N_t}$$

Als mittlerer Fehler für  $O_2$  korrig. wird  $\pm 0,03$  angegeben (NIELSEN, 1936). In der Regel wurden Doppelanalysen durchgeführt. Dadurch wurde der mittlere Fehler für die Sauerstoff-Differenz zwischen Ein- und Ausatmungsluft ungefähr 0,025. Unter den Bedingungen, unter denen ich arbeitete, beträgt die Sauerstoff-Differenz etwa 2 %. Dieser Wert ist also mit einem mittleren Fehler von etwa 1,25 % behaftet.

Die Bestimmung der Ventilationsgrösse hat einen mittleren Fehler von etwa 0,5 % (siehe auch KROGH, 1920). Der totale mittlere Fehler bei der Bestimmung der Sauerstoffaufnahme und Kohlensäureproduktion nach dieser Methode ist demnach ungefähr  $\pm 1,35$  %.

Vor und nach allen Analysen-Serien wurde die Aussenluft analysiert, um den Apparat zu kontrollieren.

#### b.) *Narkotisierte Versuchstiere.*

Die Tiere wurden mit Urethan (1,5 gr pro kg Körpergewicht) betäubt. Die Versuche wurden in gleicher Weise ausgeführt, wie es unter a beschrieben wurde, nur mit dem Unterschied, dass eine Trachealkanüle eingelegt und mit dem Atemventil verbunden wurde.

Es ist selbstverständlich mit gewissen Schwierigkeiten verknüpft, die Douglas-Methode an so kleinen Versuchstieren, wie es Meerschweinchen sind, anzuwenden. Die Methode, die hier beschrieben wurde, ist aber meiner Meinung nach gut anwendbar. Dies geht u. a. daraus hervor, dass die Atmung der Tiere nicht sichtbarlich verändert wurde, wenn die Atemmaske und das Ventil angelegt wurden. In den Versuchen mit der Atemmaske wird der schädliche Raum um etwa 2 cc vergrössert. In den Versuchen an narkotisierten Tieren dürfte keine Erhöhung des schädlichen Raumes eintreten. Auf jeden Fall sind die Verhältnisse während des ganzen Versuches jeweils konstant. Es wurden nur Versuche berücksichtigt, in denen irgend eine stenosierende Wirkung des Ventils nicht beobachtet wurde. Die Tiere sassen während der oft sehr langdauernden Versuche ruhig da. Man kann daher, meiner Meinung nach, die Veränderungen der Lungenventilation und des Gaswechsels, die während des Asthmas auftreten, sehr gut beurteilen. Die Übereinstimmung der mit dieser Methode gefundenen Werte für die Sauerstoffaufnahme mit denen, die im geschlossenen Respirationssystem beobachtet wurden, ist auffallend gut.

### 3. Temperaturmessungen.

#### a.) *An nicht narkotisierten Versuchstieren.*

In den Versuchen an nicht narkotisierten Tieren wurde die Rektal- und Hauttemperatur nach dem Prinzip gemessen, das M. NIELSEN (1938) an Versuchspersonen anwendete. Die Thermoelemente bestanden aus Konstantan und Kupfer. Das Element für die Messung der Rektaltemperatur (R) wurde aus Drähten von 0,3 mm Durchmesser gebildet, die in einen Gummischlauch eingeführt waren. Dieser wurde 5 bis 10 cm tief in das Rektum eingeschoben. Für die Messung der Hauttemperatur wurden zwei Elemente angewendet. Das eine ( $H_1$ ) bestand aus Drähten von gleichem Durchmesser wie die des Rektal-Elementes. Bei dem anderen Element wurde die eine Lötstelle, nämlich die, die dem Tiere appliziert werden sollte, von einem Konstantan-

und Kupferdraht von nur 0,1 mm Durchmesser gebildet. Diese feinen Drähte waren etwa 5 cm lang. Sie waren an ihrem Ende an Konstantan- und Kupferdrähte von 0,3 mm Durchmesser angelötet ( $H_2$ ). Auch diese Thermoelemente waren, mit Ausnahme der Lötstelle selbst, die dem Tier appliziert werden sollte, und die frei lag, in dünne Gummischläuche eingeführt. Die Lötstelle mit ihrem Schlauch wurde mit Heftpflasterstreifen auf dem Rücken des Versuchstieres befestigt, nachdem die Haare kurz geschnitten waren. Das zuletzt geschilderte Thermoelement gab etwa 1 Grad höhere Werte als das erstgenannte. Dies beruht darauf, dass die groben Drähte von der Haut Wärme ableiten. Die höheren Werte sind also richtiger. Die Leitungen der Thermoelemente gingen durch eine Röhre im Deckel der Tierkammer aus dieser heraus. Die andere Lötstelle der Thermoelemente war in eine Thermosflasche eingeführt, deren Temperatur etwa 35 Grad war, und die sich in dem gleichen Wasserbad befand, wie die Tierkammer. Die Thermosflasche war gut verschlossen. Die Wassertemperatur in ihr veränderte sich nur unbedeutend. Die Temperatur konnte auf einem Normalthermometer abgelesen und auf 0,01 Grad genau geschätzt werden. Durch einen Kupplungsschlüssel mit Quecksilberkontakt konnten die verschiedenen Thermoelemente nacheinander mit einem empfindlichen Spiegelgalvanometer in Verbindung gesetzt werden. Dieses zeigte bei meiner Versuchsmethode einen Ausschlag von 3 cm für jedes Grad Differenz zwischen den Lötstellen in dem Temperaturbereich, der gemessen wurde. Eine Veränderung von 0,01 Grad konnte gut abgelesen werden. Die Genauigkeit, mit der auf diese Weise die Temperaturen an den Versuchstieren gemessen werden konnten, betrug ungefähr 0,03 Grad.

Man darf annehmen, dass die auf diese Weise gemessene Rektaltemperatur der Bluttemperatur am nächsten liegt (u. a. CHRISTENSEN, 1931). Die Hauttemperatur dagegen wurde in diesen Versuchen dadurch, dass die Thermoelemente mit Heftpflasterstreifen befestigt wurden, von einer »Aussentemperatur« in eine »Tiefentemperatur« verwandelt, da die Bedingungen für die Wärmeabgabe seitens der betreffenden Hautstelle verändert waren. In diesen Versuchen spielt aber die genaue Hauttemperatur eine geringe Rolle. Von besonderem Interesse sind

vielmehr nur ihre Veränderungen, die während des experimentellen Asthmas auftreten. Aus dem gleichen Grunde hat es keine Bedeutung, dass die Thermoelemente für die Messung der Hauttemperatur verschieden waren. Die Hauttemperatur wurde nur an zwei Stellen gemessen. Es war daher nicht möglich, aus diesen Versuchen eine Vorstellung von der durchschnittlichen Hauttemperatur zu gewinnen. Man kann sich aber wohl eine Auffassung darüber bilden, ob grössere Veränderungen im Verhalten der Hauttemperatur während des experimentellen Asthmas vorkommen.

b.) *An narkotisierten Versuchstieren.*

An betäubten Tieren wurde nur die Rektaltemperatur bestimmt. Dies geschah so, dass ein Thermometer in das Rektum eingeführt und in passender Tiefe fixiert wurde. Um die Temperatur der narkotisierten Versuchstiere einigermassen konstant zu halten, wurden in passender Höhe über den Tieren 1 bis 2 elektrische Glühlampen angebracht. Das Versuchstier und die Lampen befanden sich unter einem Zelt. Dadurch dass die Lampen vor dem Beginn des Versuches richtig eingestellt wurden, gelang es die Temperatur der Versuchstiere ziemlich konstant zu halten. Während des Versuches selbst wurden die Wärmebedingungen nicht verändert. Auf diese Weise ist es möglich, auch an narkotisierten Tieren das Verhalten der Rektaltemperatur während der Stenoseatmung zu untersuchen.

Das Thermoelement, das für die Messung der Rektaltemperatur bestimmt war, wurde in Wasser von bekannter Temperatur kalibriert. Die anderen Thermoelemente, mit denen die Hauttemperatur gemessen wurde, wurden in Luft kalibriert. Sie wurden bei konstanter Temperatur im System in die Tierkammer gestellt. Da das Thermoelement für die Messung der Rektaltemperatur sich in einem Gummischlauch befand, entstand eine Zeitdifferenz zwischen der tatsächlichen und der von dem Thermoelement registrierten Temperatur, die etwa 2 Min. betrug (vergl. CHRISTENSEN, 1931, Seite 157).

#### 4. Blutdruckmessung, Bestimmung der Sauerstoffsättigung und des Milchsäure-Gehaltes des Blutes.

In einigen vorbereitenden Versuchen wurde bei experimentellem Asthma, das an Tieren in Urethan-Narkose ausgelöst wurde, der arterielle Blutdruck in der üblichen Weise mittels eines Quecksilbermanometers und einer Arterien-Kanüle in der Art. Carotis registriert. Zur Verhinderung der Blutgerinnung wurde Heparin benutzt.

In einigen Versuchen wurde auch die arterielle Sauerstoffsättigung an Meerschweinchen in Urethan-Narkose während des experimentellen Histamin-Asthmas bestimmt. Eine Arterien-Kanüle in Form einer T-Röhre wurde in die eine Carotis eingeführt. Durch diese Kanüle konnte das Blut ungestört von dem zentralen zum peripheren Teil der Arterie passieren. Aus dem Seitenrohr wurde unter Luftabschluss Blut entnommen. Es wurde unter Paraffin aufbewahrt. Die Bestimmungen wurden in der gewöhnlichen Weise nach VAN SLYKE ausgeführt. Auch in diesen Versuchen wurde Heparin zur Verhinderung der Blutgerinnung benutzt.

Auf die gleiche Weise wurde auch Blut zur Untersuchung des Milchsäure-Gehaltes entnommen. Dieser wurde nach LEHMANN (1938) und nach MÜLLER und MUNTZ (1938) bestimmt. Der Milchsäure-Gehalt des Blutes und in einigen Fällen auch der Brenztraubensäure-Gehalt wurde außerdem auch an nicht narkotisierten Tieren untersucht, die für die Versuche über das Verhalten der Gewebsatmung benutzt wurden. Der Brenztraubensäure-Gehalt wurde nach KLEIN (1941) bestimmt. Die Versuchstiere wurden durch Nackenschlag getötet, die Carotiden geöffnet und das Blut aufgefangen. Die Milchsäure-Analysen wurden im Zentrallaboratorium des Sahlgren'schen Krankenhauses in Göteborg, die Bestimmungen der Brenztraubensäure im Rockefeller-Laboratorium der Medizinischen Klinik in Lund durchgeführt. Ich möchte an dieser Stelle den Herren Prof. LEHMANN, Doz. HOLMBERG und Dr. STENSTAM, die freundlicherweise diese Untersuchungen ausführten, danken.

## 5. Untersuchung der Gewebsatmung.

### a.) *Methode von Warburg.*

Was diese Methode selbst betrifft, möchte ich auf KREBS (1929), und DICKENS (1940) verweisen. Ich benutzte die Apparatur, die von AHLGREN (1929) angegeben wurde. Die Rezipienten waren genau mit Quecksilber ausgewogen und hatten im allgemeinen ein Volumen von etwa 16.5 cc. Als Versuchsmedium benutzte ich Phosphat-Puffer, der nach zahlreichen Untersuchungen ein besonders geeignetes Milieu für Muskelschnitte aber auch für andere Gewebe ist (THUNBERG, 1909, ALWALL, 1935, KREBS und EGGLESTON, 1938).

Ich benutzte Phosphat-Puffer nach SØRENSEN. Diese Lösung hatte eine Endkonzentration von m/18. Ihr  $p_{\text{H}}$  war 7.15. Es zeigte sich, dass die Lösung in dieser Konzentration ein genügend starkes Puffervermögen besass. Dies stimmt mit Versuchen von ALWALL (1935) überein, der zeigte, dass ein Phosphat-Puffer von m/20 ein genügend starkes Puffervermögen hatte. Nach Beendigung der Versuche wurde in der Regel das  $p_{\text{H}}$  der in den Rezipienten befindlichen Lösungen kontrolliert. Bei der Untersuchung mit Glaselektroden zeigte sich, dass diese Lösungen ein  $p_{\text{H}}$  hatten, das gar nicht oder nur unwesentlich von dem des Phosphat-Puffers abwich. Wurden Substanzen, deren  $p_{\text{H}}$  von dem des Phosphat-Puffers verschieden war, in solchen Mengen zugesetzt dass sie das  $p_{\text{H}}$  des Puffers verschieben könnten, so wurden die Zusätze vorher neutralisiert. Um einen orientierenden Überblick über Veränderungen im  $p_{\text{H}}$  zu erhalten, wurde in manchen Fällen Indikatorpapier (Lyphan L 668) benutzt. Dies war möglich, da es sich stets um gut gepufferte Lösungen handelte. Das genaue  $p_{\text{H}}$  der benutzten Phosphat-Puffer wurde mittels Chinhydronelektrode bestimmt.

Die Versuchstiere wurden durch Nackenschlag getötet, und die Carotiden möglichst schnell geöffnet, damit die Tiere sich verbluten konnten. Die Organe, die untersucht werden sollten, wurden möglichst rasch entnommen und in eisgekühlte Gläser gelegt. Nachdem die Gewebe möglichst vom Blut befreit und von Fascien und ähnlichen Anhängseln freipräpariert waren, wurden

sie innerhalb von 4 Minuten auf einem eisgekühlten Uhrglas klein geschnittenen. Nachdem die Gewebe klein geschnitten waren, wurden sie eisgekühlt in gut verschlossenen Gläsern aufbewahrt. Je 200 mg wurden abgewogen und in die verschiedenen Rezipienten gebracht, die mit 2,5 cc Suspensionsflüssigkeit beschickt waren. Diese wurden zugekorkt und eisgekühlt aufbewahrt, bis sie auf die Manometer gesetzt wurden, unmittelbar bevor letztere ins Wasserbad kamen. In der Regel geschah dies genau eine Stunde, nachdem das Tier getötet worden war. Zur Absorption der Kohlensäure war in dem Mittel-Napf der Rezipienten 0,3 cc 1/n KOH. Die Temperatur des Wasserbades wurde bei genau 37 Grad gehalten (auf 0,1 Grad exakt).

In einer kleineren Versuchsreihe wurde die anaerobe Glykolyse manometrisch bestimmt. Bezuglich der Methode verweise ich auf KREBS (1929), DICKENS (1940) und BOYLAND (1941). Für diese Versuche wurde eine Glykose und Bikarbonat enthaltende Ringerlösung in der Zusammensetzung angewendet, wie sie bei DICKENS beschrieben wurde. Die Versuche wurden in einer Stickstoff-Atmosphäre mit 5 % Kohlensäure-Gehalt durchgeführt. Diese Gasmischung, die in Gasbomben zu erhalten war, hatte aber ausserdem einen Sauerstoff-Gehalt von 0,2 %. Die Gasmischung wurde über rotglühendes Kupfer geleitet, das sich in einer mit einer Wärmespirale versehenen Quarzröhre befand. Hierdurch wurde der Sauerstoff-Gehalt auf etwa 0,05 % herabgesetzt. Der so gewonnene Sauerstoff wurde zur Füllung der Respirimeter benutzt. Auch in diesen Versuchen wurde Gewebe-Brei angewendet, obgleich die manometrische Bestimmung der anaeroben Glykolyse bei der Benutzung von Brei weniger sicher ist, als bei der von Gewebs-Schnitten (BOYLAND, 1941). Die betreffenden Versuche wurden aber nur zu dem Zweck angestellt, zu ermitteln, inwieweit grössere Unterschiede in der anaeroben Glykolyse während des experimentellen Asthmas zu beobachten wären.

#### b.) *Methylenblau-Methode.*

Die Versuche mit dieser Methode wurden parallel mit den Warburg Versuchen durchgeführt. Hierfür wurden unmittelbar nach dem Beginn des Warburg-Versuches 200 mg Gewebe ab-

gewogen. Bezuglich der Methode verweise ich auf AHLGREN (1925 und 1934) und ALWALL (1935).

Es wurden sowohl gewöhnliche Thunberg-Röhren wie auch die von AHLGREN modifizierten T-förmigen Typen benutzt. In den Thunberg-Röhren wurde eine Flüssigkeitsmenge von insgesamt 1,3 cc und 0,2 gr Gewebe angewendet, in den Ahlgren-Röhren eine Flüssigkeitsmenge von insgesamt 5 cc und 0,2 gr Gewebe. Als Suspensionslösung wurde auch hier ein Phosphat-Puffer ( $p_H = 7,15$ ) benutzt. In den Thunberg-Röhren war der Puffer m/7,5, in den Ahlgren-Röhren m/18. In der Regel kamen 1000 γ Methylenblau zur Anwendung, in einem Teil der Versuche auch 1250 und 1500 γ.

Die Evakuierung wurde durch eine Vakuumpumpe bewirkt, die bis auf 2 mm Quecksilber herunter ging. Die Temperatur im Wasserbad war 37 Grad.

Die Kontrolle des  $p_H$  wurde auch in diesen Versuchen mittels Glaselektrode häufig vorgenommen.

## 6. Bestimmung der Cozymase.

Die Extraktion für die Bestimmung des Cozymase-Gehaltes in den Geweben erfolgte nach EULER, SCHLENK, HEIWINKEL und HÖGBERG (1938). Für die Bestimmung der gesamten Cozymase-Menge wurde demgemäß mit Wasser extrahiert, für die Bestimmung der Dihydrocozymase und der oxydierten Cozymase mit m/20 NaOH, bezw. mit m/20 HCl.

Die Tiere wurden in der gewöhnlichen Weise durch Nackenschlag getötet. Man liess sie verbluten und entnahm dann möglichst rasch die Organe, die in eisgekühlte Gläser gelegt wurden. 0,5 gr Gewebe (bezw. 1 gr für die Bestimmung der Dihydrocozymase und der oxydierten Cozymase) wurden schnell abgewogen und während 5 Sekunden klein geschnitten. Dann kamen sie in Zentrifugengläser, die 1 cc Extraktionsmittel enthielten und in einem kochenden Wasserbad standen. Als Extraktionsmittel wurden doppelt destilliertes Wasser, m/15 Phosphat-Puffer  $p_H$  6,3, m/20 HCl und m/20 NaOH angewendet. Nach 4 Minuten wurden die Gläser rasch abgekühlt. Man liess sie

dann  $\frac{1}{2}$  Stunde in kaltem Wasser stehen. Danach wurde zentrifugiert und das Extraktionsmittel dekantiert. Darauf wurde 1 cc neues Extraktionsmittel zugesetzt, worauf die Gewebsmassen im Zentrifugenglas mit einer schmalen Schere sorgfältig klein geschnitten wurden. Das Röhrchen blieb dann 15 Minuten stehen. Nach dieser Zeit wurde wieder zentrifugiert. Das Extraktionsmittel wurde dekantiert und mit dem ersten Extrakt vereinigt. Wenn das Extraktionsmittel aus HCl oder NaOH bestand, wurde der Extrakt neutralisiert. Dann wurde der Extrakt auf 3 cc verdünnt. Die Neutralisation und Verdünnung wurde im allgemeinen 2 Stunden, nachdem das Versuchstier getötet worden war, vorgenommen. Darauf wurde das Zentrifugenträgerröhrchen im Eisschrank bis zum Beginn des Gärungsversuches aufbewahrt. Dieser wurde in der Regel 3 Stunden nach der Tötung des Tieres durchgeführt. Für den Gärungsversuch wurden 1 cc Extrakt angewendet.

Die quantitative Bestimmung der extrahierten Cozymase erfolgte im Gärungsversuch nach MYRBÄCK (1928 und 1937). Die Vergärungen wurden jedoch in einem Warburg-Apparat durchgeführt. Bezuglich der Methode verweise ich auf SCHLENK (1941). Die Vergärung wurde in Luftatmosphäre vorgenommen. Ich wendete Trockenhefe und Apoczymase an, die aus Unterhefe hergestellt wurde. Letztere wurde mir bereitwilligst von der Hamburger Brauerei in Stockholm zur Verfügung gestellt. Die Gär-mischung hatte folgende Zusammensetzung: 200 mg Apoczymase, 50 mg Glykose, 0,30 cc Hexosediphosphat, etwa 5 % -ig als Natriumsalz, 0,2 mg MgSO<sub>4</sub>, 0,2 mg MnSO<sub>4</sub>, 0,30 cc Phosphat-Puffer, m/2,5, pH 6,3. Gesamtmenge 3 cc.

Die Extrakte aus den Geweben wurden im Gärungsversuch mit einem Cozymase-Präparat verglichen, das von Herrn Prof. H. VON EULER freundlicherweise dem Institut zur Verfügung gestellt wurde. Bei einer Aktivitätsprüfung, die im Institut von Prof. VON EULER im Februar 1942 vorgenommen wurde, erwies sich dies Präparat als etwa 98 % -ig. Es enthielt ungefähr 1 % Adenylsäure. Dieses Cozymase-Präparat wird Cozymase I genannt. Außerdem wendete ich für meine Versuche ein von Doz. HOLMBERG hergestelltes Präparat an, das ebenfalls freundlicherweise zu meiner Verfügung gestellt wurde. Dies Präparat

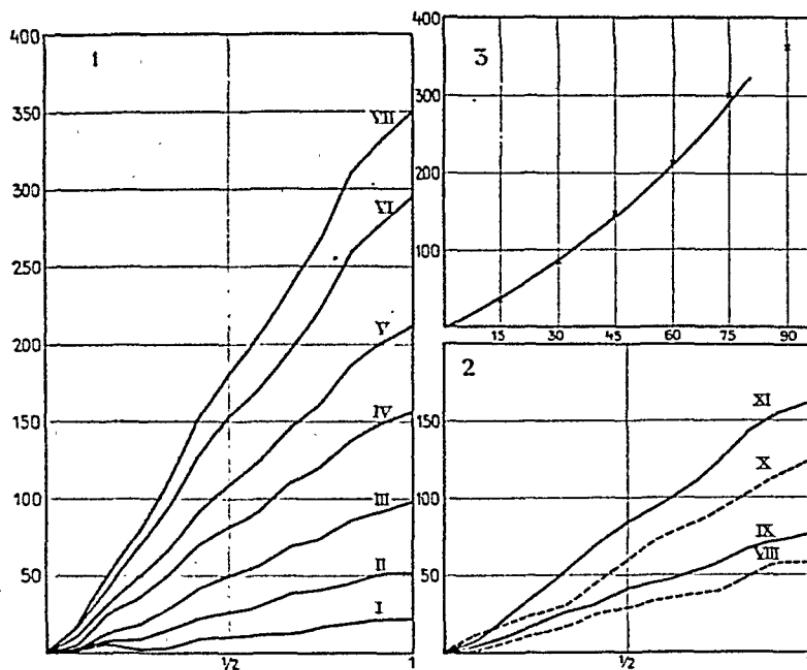


Abb. 7. Gärungsversuch vom 5.2.1943. Apozymase VI.

1. Ordinate: cmm  $\text{CO}_2$ . Abszisse: Zeit in Stunden.  
I. 0-Gärung. ohne Cozymase-Zusatz. II. Zusatz von 15 γ, III. von 30 γ, IV. von 45 γ, V. von 60 γ, VI. von 75 γ. VII. von 90 γ Cozymase I.
2. Ordinate und Abszisse wie bei 1.  
VIII. Zusatz von 100 γ, X. von 200 γ Cozymase III.  
IX. Zusatz von 50 γ, XI. von 100 γ Cozymase II.
3. Ordinate: cmm  $\text{CO}_2$ . Abszisse: γ Cozymase I.

wird als Cozymase II bezeichnet. Ein drittes Präparat stellte ich selbst her, Cozymase III. Cozymase II und III wurden nach der Methode von WILLIAMSON und GREEN (1940) dargestellt. Im Gärungsversuch zeigte sich, dass sich die Aktivität der oben genannten Präparate wie 1 : 0,41 : 0,17 verhielt.

Um den Cozymase-Gehalt in den Extrakten feststellen zu können, musste ein Vergleich mit bekannten Mengen Cozymase vorgenommen werden. Dies geschah nach MYRBÄCK so, dass in 5-Minuten-Perioden die Kohlensäure-Entwicklung verschieden grosser Mengen von Cozymase I bestimmt wurde. Ein derartiger Versuch ist in Abbildung 7 Nr. 1 dargestellt. Die erhaltenen Werte wurden in eine Kurve eingezeichnet, die die produzierten

Kohlensäure-Mengen als Ordinate und die Zeit vom Beginn der Gärung an als Abszisse enthielt. Zwischen der 30. und 60. Minute wurde eine gerade Linie durch möglichst viele Punkte für eine gegebene Cozymase-Konzentration gezogen. Auf dieser Linie wurde die Kohlensäure-Produktion während  $\frac{1}{2}$  Stunde abgelesen. Dann wurde der Wert für eine Stunde berechnet. Aus der so gefundenen Kohlensäure-Entwicklung pro Stunde wurde eine Vergleichskurve für verschiedene grosse Mengen von Cozymase gezeichnet, wie es aus Abbildung 7 Nr. 3 hervorgeht. Nachdem in entsprechender Weise die Kohlensäure-Entwicklung für die verschiedenen Extrakte im Gärungsversuch bestimmt worden war, erhielt man aus der Vergleichskurve ihre Cozymase-Mengen. Die Gärungsversuche, die der Vergleichskurve und der Untersuchung der Extrakte selbst zu Grunde liegen, müssen mit der gleichen Apozymase durchgeführt werden. Sie müssen ferner zeitlich möglichst nahe beieinander liegen, ihr zeitlicher Abstand soll am besten nicht mehr als zwei Wochen sein, da sich die Apozymase so lange als ausreichend konstant erwies.

Im Zusammenhang mit allen Vergärungsversuchen wurden Additionsversuche ausgeführt, in denen ein unbekannter Extrakt zusammen mit einer bekannten Menge Cozymase geprüft wurde, um zu kontrollieren, dass die Extrakte keine gärungshemmenden Stoffe enthielten. Eine solche hemmende Wirkung hat sich aber niemals gezeigt. Als bekannte Cozymase-Menge wurde in diesen Versuchen ein Kochsaft aus Trockenhefe benutzt. Dieser wurde für jeden Versuch in gleicher Weise hergestellt: 0,25 gr Trockenhefe und 5 cc doppelt destilliertes Wasser wurden 2 Minuten gekocht und dann filtriert. Die Cozymase-Menge in diesem Kochsaft wurde in der oben angegebenen Weise bestimmt. In einer Versuchsserie, die später näher zu besprechen ist, wurden die folgenden, in Tabelle 2 wiedergegebenen Werte für den Cozymase-Gehalt des Kochsaftes gefunden. Sie können auch als Beleg für die Sicherheit der von mir benutzten Gärungsmethode dienen. Zu allen Versuchen wurde Apozymase VI verwendet.

Was die Genauigkeit der Extraktionsmethode betrifft, so betonen VON EULER und seine Mitarbeiter (1938), dass auf sie vor allem zwei verschiedene Faktoren einwirken. Zum Ersten bekommt man stets deshalb geringere Cosymase-Mengen, als die

Tabelle 2.

Datum des Versuches	Kochsaft in cc	Gefundene Menge Cozymase in γ
26. 1. 43.	0,25	20
28. 1. 43.	0,25	19
29. 1. 43.	0,25	20
1. 2. 43.	0,5	42
2. 2. 43.	0,5	39

die tatsächlich in den Organen vorhanden sind, weil ein Teil der Cozymase bei der notwendigen Erhitzung zerstört wird und weil die Extraktion nicht vollständig ist. Die genannten Verfasser geben die Grösse des Defizits, das auf diese Weise zu Stande kommt, mit 20 bis 40 % an. Zum Andern ist die Methode selbst mit einem Fehler von etwa  $\pm 20\%$  behaftet.

Da es für mich nur darauf ankam, den Cozymase-Gehalt in den Geweben von normalen Versuchstieren mit dem in den Geweben von Tieren, die Asthma hatten, zu vergleichen, so war es von geringer Bedeutung, dass die verglichenen Werte sämtlich prozentual ungefähr in gleichem Masse zu niedrig waren. Der mittlere Fehler für die einzelnen Bestimmungen liegt bei der von mir angewendeten Methode zur Extraktion der Cozymase aus den Geweben von verschiedenen Versuchstieren und zur Bestimmung der Cozymase-Mengen, die sich in den Extrakten befinden, bei ungefähr  $\pm 15\%$ . Dies gilt für Normalmaterial, bei der Extraktion mit Wasser wie mit Phosphat-Puffer. Vergleiche ferner Tabelle XX.

In der oben zitierten Arbeit von v. EULER wurde der Cozymase-Gehalt in der Leber der Ratte bestimmt. Es wurden 215 γ pro gr frisches Gewebe gefunden. Eine Angabe über den Cozymase-Gehalt der Leber beim Meerschweinchen konnte ich in der Literatur nicht finden. In meinen Versuchen erhielt ich bedeutend höhere Werte, bis zu 500 γ pro gr frisches Gewebe. Dies könnte darauf beruhen, dass entweder die Meerschweinchen einen grösseren Cozymase-Gehalt in ihrer Leber haben, oder darauf, dass die von mir angewendeten Cozymase-Präparate seit der Bestimmung ihrer Aktivität schwächer geworden waren. Die absoluten Werte haben aber, wie bereits früher betont, in dem mich hier interessie-

renden Zusammenhang keine besondere Bedeutung. Es kam hier nur darauf an, den Cozymase-Gehalt, vor allem in der Leber, von normalen und von asthmatischen Versuchstieren zu vergleichen.

### 7. Angewendete Präparate.

- 1.) Phosphat-Puffer nach SÖRENSEN (Na<sub>2</sub>HPO<sub>4</sub> + 2H<sub>2</sub>O, KH<sub>2</sub>PO<sub>4</sub>).
- 2.) Methylenblau, medizinisch-chemisch rein und chlorzinkfrei. »Merck». (C<sub>16</sub>H<sub>12</sub>N<sub>2</sub>SCl + 3 H<sub>2</sub>O). Bei den Angaben über die Konzentration wurde diese aus dem wasserhaltigen Präparat berechnet.
- 3.) Histamindihydrochlorid (C<sub>5</sub>H<sub>9</sub>N<sub>3</sub> + 2 HCl) »Merck« (4370, 2092, 3249 T).
- 4.) d-, L-Milchsäure, Laetic acid, analytical B. D. H., London. Die in den Versuchen angegebenen Konzentrationen sind solche von d-Milchsäure. Das Präparat wurde mit NaOH neutralisiert.
- 5.) Bernsteinsäure. Acid.succinicum albiss. pro analysi »Merck«. Das Präparat wurde mit NaOH neutralisiert.
- 6.) Cozymase-Präparate. Diese wurden in Abschnitt 6 beschrieben.
- 7.) Adenylsäure. Adenylsäure »Merck« (42690), adenylic acid B.D.H. (363712).
- 8.) Adenosin B.D.H. (587042/38714).
- 9.) Adenin »Merck« (W 41372, 838, 1032).
- 10.) Nicotinsäureamid, »Merck« (W 31118, 6755, 20594), Hoffmann-La-Roche (15792, 5206).
- 11.) Cocarboxylase (ca. 80 % ig). Dies Präparat wurde mir von Herrn Phil. Dr. E. H. LUNDIN, Kärnbolaget, Stockholm, freundlicherweise zur Verfügung gestellt.
- 12.) Natriumhexosediphosphat. Das Präparat wurde aus »glukofos Astra», das Calciumhexosediphosphat mit einem deklarierten Gehalt von 17 % Calcium und 30 % P<sub>2</sub>O<sub>5</sub> ist, durch Umsetzung mit Natriumoxalat dargestellt. Dabei wurden etwa 15 % weniger als die berechnete Menge Oxalat verwendet, um einen Oxalat-Überschuss in der Lösung

sicher zu vermeiden. Die Lösung wurde 24 Stunden in der Schüttelmaschine geschüttelt und dann mehrfach, zuletzt warm, filtriert. Die so gewonnene Lösung wurde steril aufbewahrt. Diese Lösung von Natriumhexosedi-phosphat enthielt bei der Prüfung in jedem Fall weniger als 1 mg Oxalat auf 5 cc. Da den verschiedenen Gärungsansätzen 0,3 cc dieser Lösung zugesetzt wurde, war die Konzentration des Oxalates in der Gärmbewirkung auf jeden Fall kleiner als m/6000. Nach BOYLAND (1941, Seite 2263) hemmt Oxalat die Glykolyse um 50 % bei einer Konzentration von m/200.

### 8. Die Auslösung des Asthmas.

Experimentelles Asthma wurde teils durch Histamin, teils durch das homologe Antigen bei vorher sensibilisierten Tieren hervorgerufen. Als Antigen wurde fast ausschliesslich Homoserum angewendet. Die Stärke der zu verwendenden Lösungen musste in jedem einzelnen Versuch ausprobiert werden. Häufig war es mit Schwierigkeiten verbunden, gerade die Konzentration zu finden, die ein ausreichendes und gleichmässiges Asthma bewirkte. Die Histamin-Lösungen wurden zwischen 0,1 und 5 pro mille variiert. Das Serum wurde teils unverdünnt, teils bis zu 10 fach mit physiologischer Kochsalzlösung verdünnt angewendet.

### 9. Versuchstiere.

Für alle Versuche wurden ausschliesslich Meerschweinchen benutzt. Für die Respirationsversuche wurden in der Regel grössere Meerschweinchen von 500 bis 700 gr, ausschliesslich männliche Tiere, verwendet. Nach KROGH und LINDBERG (1931 und 1932) ist nämlich der Sauerstoffverbrauch bei grösseren männlichen Meerschweinchen gleichmässiger. Diese gewöhnen sich ausserdem rascher an die Apparatur. Durch Vorversuche mussten sich die Tiere stets an die Apparatur gewöhnen, bevor sie zu den Versuchen selbst verwendet wurden. Bei manchen Tieren wurde das Asthma mehrere Male ausgelöst. Für die Versuche

über die Gewebsatmung wurden auch kleinere Tiere verwendet, aber in jeder Versuchsreihe ungefähr gleichgrosse Tiere, denn jüngere Tiere haben häufig intensivere oxydative Prozesse in ihren Geweben, als ältere Tiere. Dies wurde u. a. von ABDERHALDEN und WERTHEIMER (1922) nachgewiesen. In der Regel wurden nur männliche Tiere angewendet. Ungefähr 10 Tiere wurden gleichzeitig vom Tierzüchter angeschafft. Diese hatten sich zunächst mindestens 2 Wochen, oft länger, an die Verhältnisse im Tierstall des Institutes zu gewöhnen. An einer derartigen Serie von Versuchstieren wurden abwechselnd Normalversuche und Asthmaversuche durchgeführt, um auf diese Weise möglichst kennzeichnende Werte zu gewinnen. In der Regel wurden die Tiere von dem gleichen Tierzüchter bezogen, damit das Material möglichst einheitlich war.

Für die Respirationsversuche blieben die Tiere 12 Stunden von Beginn des Versuches ohne Futter. Das Gleiche war der Fall in manchen der Versuchsreihen, in denen die Gewebsatmung untersucht wurde. In anderen dieser Versuchsreihen erhielten die Tiere bis 6 Stunden vor dem Versuch ihr Futter. Bis zum Beginn des Versuches hatten sie stets Zugang zum Wasser.

## KAP. IV.

### Respirationsversuche im geschlossenen System.

In diesem Kapitel werden Versuche mit kontinuierlicher Registrierung des Sauerstoffverbrauches beim experimentellen Asthma, das durch Histamin wie auch auf anaphylaktischem Wege ausgelöst wurde, dargestellt. Ausser dem Sauerstoffverbrauch wurde in der Regel die Rektal- und Hauttemperatur der Versuchstiere fortlaufend aufgezeichnet. Der Allgemeinzustand der Tiere wurde durch ein Glasfenster im Deckel des Tierkastens beobachtet. Auf diese Weise konnte auch der Schweregrad des Asthmas beurteilt werden. Die Atemfrequenz wurde durch direkte Zählung bestimmt. Zwischen den beiden oben genannten Formen des experimentellen Asthmas konnte kein prinzipieller Unterschied konstatiert werden.

Der Schweregrad des Asthmas wurde durch die Ziffern 1 bis 5 bezeichnet. 1. Leichtes Asthma: Die Atemzüge waren deutlicher markiert als normal und etwas erschwert. 3. Mittelschweres Asthma: Deutlich erschwerete Ausatmung, regelmässige Atemfrequenz. 5. Schweres Asthma: Die stärkste Atemerschwerung, die die Versuchstiere aushalten können. Bei der Ausatmung, die bedeutend verlängert ist, können die Tiere die Luft nur mit grosser Schwierigkeit herauspressen. Die Atempausen sind verschieden lang. Hierbei besteht dauernd die Gefahr, dass die Tiere ersticken könnten, wenn sie nicht im Stande waren, die erschwerete Atemarbeit fortzusetzen. 2 und 4 bezeichnen die Schweregrade eines Asthmas, das zwischen den oben dargestellten Grenzen liegt. Wenn auch die Grundlage der Beurteilung des Grades des Asthmas rein subjektiv war, konnte doch auf diese Weise eine ganz gute Vorstellung vom Zustande der Versuchstiere gewonnen werden.

*Der Allgemeinzustand der Versuchstiere.* Die meisten Tiere sitzen, während der Atmungserschwerung ganz ruhig. Nach einem schwereren Asthma-Anfall, der längere Zeit anhielt, sehen die Tiere matt und schlapp aus, ihre Atemzüge wirken ermüdet. Man hat den Eindruck, dass die Tiere gewissermassen Schwierigkeiten haben, die Atmungsbehinderung zu bewältigen. Oft halten sie den Kopf nach oben und etwas nach hinten geneigt, wahrscheinlich, um auf diese Weise die Arbeit der auxiliären Atemmuskulatur zu erleichtern. Der Kopf wird mit jedem Atemzug vor und zurück bewegt, und die Atembewegungen sind auch im übrigen nicht auf den Brustkorb beschränkt, sondern der ganze Körper nimmt sozusagen an ihnen Teil. Nicht selten zittern die Tiere ein wenig. In den schwersten Fällen liegt der Unterkörper schlapp da, während der Kopf andauernd aufrecht gehalten wird. Nachdem ein Asthma längere Zeit bestanden hat, kann sich das Versuchstier manchmal auf die Seite legen. Man hat den Eindruck, dass die Tiere meist ein schweres anaphylaktisches Asthma besser ertragen können, als ein Histamin-Asthma von anscheinend gleichem Grad.

*Die Atemfrequenz.* Wenn man die geeignete Stärke der Lösung ausprobiert, die angewendet werden soll, um das Asthma auszulösen, verfährt man zweckmässigerweise so, dass man mit schwächeren Konzentrationen beginnt und diese dann allmählich erhöht. Der erste Effekt, den man dabei an dem Versuchstier beobachten kann, ist eine deutliche Erhöhung der Atemfrequenz, ohne dass dabei die Atmung erschwert ist. Wenn die angewendete Konzentration gerade den Schwellenwert überschreitet, der notwendig ist, um eine Veränderung der Atmung auszulösen, so kann die Atemfrequenz lange Zeit erhöht bleiben (siehe Abb. 11 und 12). Bei etwas stärkeren Konzentrationen nimmt in der Regel die Atemfrequenz mit steigendem Schweregrad des Asthmas ab (siehe Abb. 8, 9 und 10). Bei schwerem Asthma kann die Atemfrequenz unterhalb des Normalwertes liegen. Wenn man mit der Inhalation der Lösung, die das Asthma hervorruft, aufhört, steigt die Atemfrequenz wieder an, gleichzeitig damit, dass die vorher erschwerte Atmung allmählich wieder leichter wird. Die Frequenzerhöhung übertrifft dabei häufig die initiale Frequenzzunahme im Beginn des Asthmas.

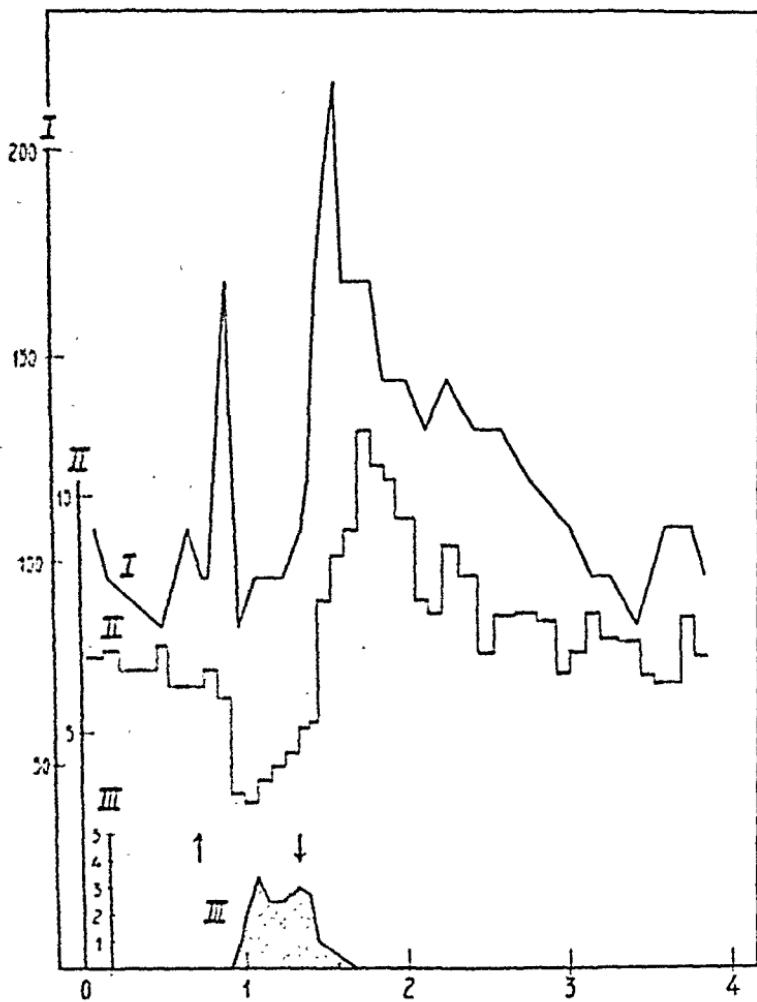


Abb. 8. Versuch vom 13. 12. 1939. Geschlossenes Respirationssystem mit atmosphärischer Luft. Gewicht des Versuchstieres 750 gr. Asthma durch 1.25 %ige Histaminlösung hervorgerufen. Die Pfeile markieren den Beginn und das Ende der Inhalation der Histaminlösung.

Allgemeinzustand des Tieres: Das Versuchstier verhielt sich während der ganzen Zeit ruhig.

- I. Atemfrequenz pro Minute.
- II. Sauerstoffaufnahme in cc pro Minute (reduziert auf 0°, 760 mm Hg und Trockenheit).
- III. Grad des Asthma (1. Leichtestes Asthma, 3. mittelschweres Asthma, 5. schweres Asthma).

Abszisse: Zeit in Stunden.

(siehe Abb. 8 und 9). Allmählich sinkt dann die Frequenz wieder zur Norm. Die Kurve der Atemfrequenz hat demnach einen zweigipfligen Verlauf.

Die Veränderungen in der Atemfrequenz treten in der Regel rascher auf, wenn das Asthma durch Histamin ausgelöst wird, als wenn man es durch Anaphylaxie hervorruft. Andererseits hält die Erschwerung der Atmung bei anaphylaktischem Asthma länger an, als bei dem Histaminasthma, nachdem die Einatmung der Asthma bewirkenden Lösung ausgeschaltet wurde.

Die erste Frequenzsteigerung kann man wahrscheinlich als eine Folge der sich allmählich immer mehr entwickelnden Verengerung der Luftwege ansehen. Man darf annehmen, dass sie entsteht, indem der Organismus den auftretenden Atmungswiderstand zu überwinden sucht. Bei schwererer Stenoseatmung aber verhindert diese die Frequenzerhöhung, und die Frequenz nimmt umgekehrt ab, u. a. infolge der Verlängerung der Ausatmung. Wenn nach einem schwereren Asthma die Atmungsbehinderung nachlässt, wird die Frequenz wieder im gleichen Masse erhöht, wie sich die Enge der Atemwege vermindert. Dieser zweite Gipfel der Atemfrequenz wird wahrscheinlich auch dadurch verursacht, dass der Organismus eine grösse Lungenventilation braucht, um den erhöhten Sauerstoffbedarf zu befriedigen und um u. a. die Sauerstoffschuld zurückzuerstatten, die vorher während des Asthmas entstanden ist, wie es weiter unten geschildert werden wird.

*Die Sauerstoffaufnahme.* Die Sauerstoffaufnahme ist bei leichteren Atembeschwerden um 5 bis 20 % gesteigert. Diese Erhöhung kann während des ganzen Versuches andauern (Abb. 12) oder auch nur während einer längeren Zeit (Abb. 11). Oft sieht man aber auch eine Steigerung nur im Beginn des Asthma-Anfallen, bevor die Atmungsbehinderung allzu ausgesprochen wird (Abb. 10 und 13). Da die Erhöhung der Sauerstoffaufnahme stärker ist, als es der Steigerung der Körpertemperatur entspräche, haben wir bei leichtem Asthma eine vergrösserte aerobe Kalorienproduktion im Vergleich zu der Kalorienproduktion unter Standardbedingungen. Diese initiale Steigerung der Sauerstoffaufnahme ist bei erhöhtem Sauerstoff-Gehalt im System deutlicher ausgeprägt, als wenn das System mit atmosphärischer

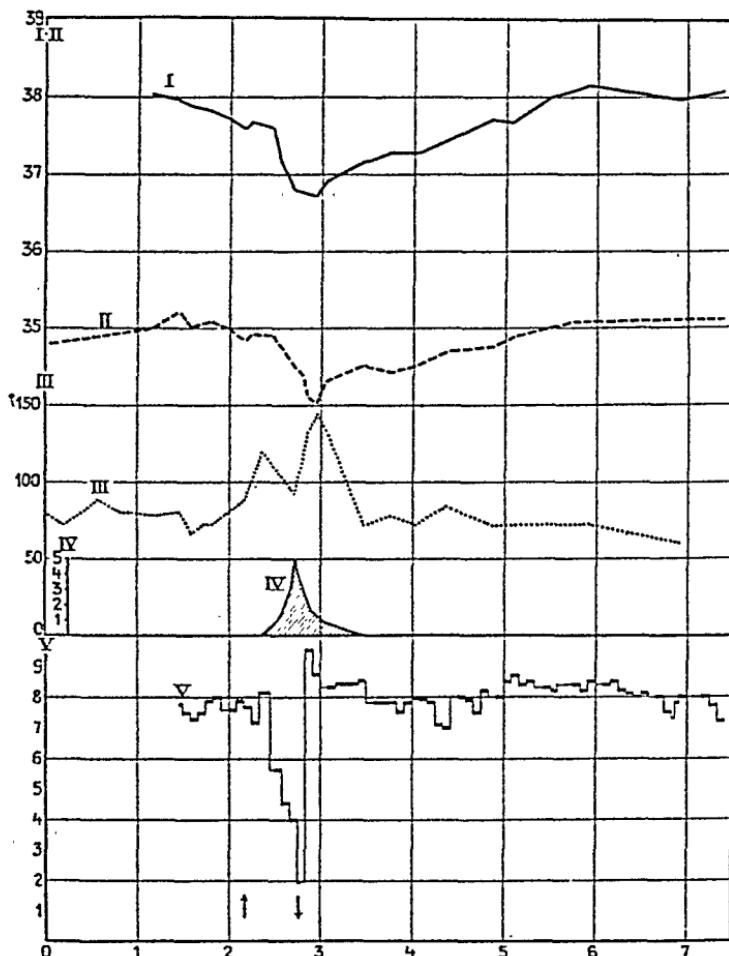


Abb. 9. Versuch vom 28.3.1940. Geschlossenes Respirationssystem mit atmosphärischer Luft. Gewicht des Versuchstieres 550 gr. Asthma durch 5 %oige Histaminlösung hervorgerufen.

Allgemeinzustand des Versuchstieres: Ruhig während des ganzen Versuches.

- I. Rektaltemperatur in Grad Celsius.
- II. Hauttemperatur in Grad Celsius.
- III. Atemfrequenz pro Minute.
- IV. Grad des Asthmas.
- V. Sauerstoffaufnahme in cc pro Minute (reduziert).

Abszisse: Zeit in Stunden.

Luft gefüllt ist. In Abb. 9 sieht man daher nur eine Andeutung der initial vergrösserten Sauerstoffaufnahme, da in diesem Versuch das System atmosphärische Luft enthielt. Manchmal beginnt das Asthma sehr rasch und wird bald ziemlich schwer. In diesen Fällen findet man keine Steigerung der Sauerstoffaufnahme im Beginn des Asthma-Anfalles (Abb. 8).

Bei schwererem Asthma sinkt die Sauerstoffaufnahme immer nach einer gewissen Zeit, gleichgültig ob das System mit atmosphärischer Luft oder mit 90 % Sauerstoff gefüllt wurde. Dabei ist die Senkung bedeutend stärker ausgesprochen, wenn das System Luft (Abb. 8 und 9), als wenn es Sauerstoff enthält (Abb. 10 und 13). Bei erhöhtem Sauerstoff-Gehalt im System können die Versuchstiere erheblich leichter ein schweres Asthma ertragen. Die Herabsetzung der Sauerstoffaufnahme kann in Luft 50 bis 75 % betragen, während ihre Verminderung bei entsprechendem Asthma in Sauerstoff nur etwa 25 % ausmacht.

In der Nachperiode verhält sich die Sauerstoffaufnahme in verschiedenen Fällen different. War das Asthma leicht und ging während der ganzen Zeit mit einer unbedeutenden Erhöhung der Sauerstoffaufnahme einher, so wird letztere in dem Augenblick wieder normal, in dem die Atmung zur Norm zurückkehrt. Man findet hier keine Erhöhung der Sauerstoffaufnahme in der Nachperiode (Abb. 12). In manchen Fällen, besonders bei erhöhtem Sauerstoff-Gehalt im System, kann man bei etwas schwererem Asthma im ersten Teil der Nachperiode eine Steigerung der Sauerstoffaufnahme um 20 bis 30 % sehen, obgleich letztere während des ganzen Asthmas um etwa 10 % über dem Normalwert lag. Dies muss man wahrscheinlich so deuten, dass die Erhöhung der Sauerstoffaufnahme während des Asthma-Anfalles doch nicht ausreichte, um den Bedarf unter diesen Verhältnissen zu decken. War das Asthma so schwer, dass die Sauerstoffaufnahme während des Anfalles unter den Standard-Wert herabgesetzt war, so sieht man in der Nachperiode meist eine Erhöhung der Sauerstoffaufnahme über die Norm. Die Vergrösserung der Sauerstoffaufnahme im ersten Teil der Nachperiode ist aber bei den verschiedenen Fällen sehr different. Manchmal übertrifft so die Zunahme der Sauerstoffaufnahme in der Nachperiode mehr

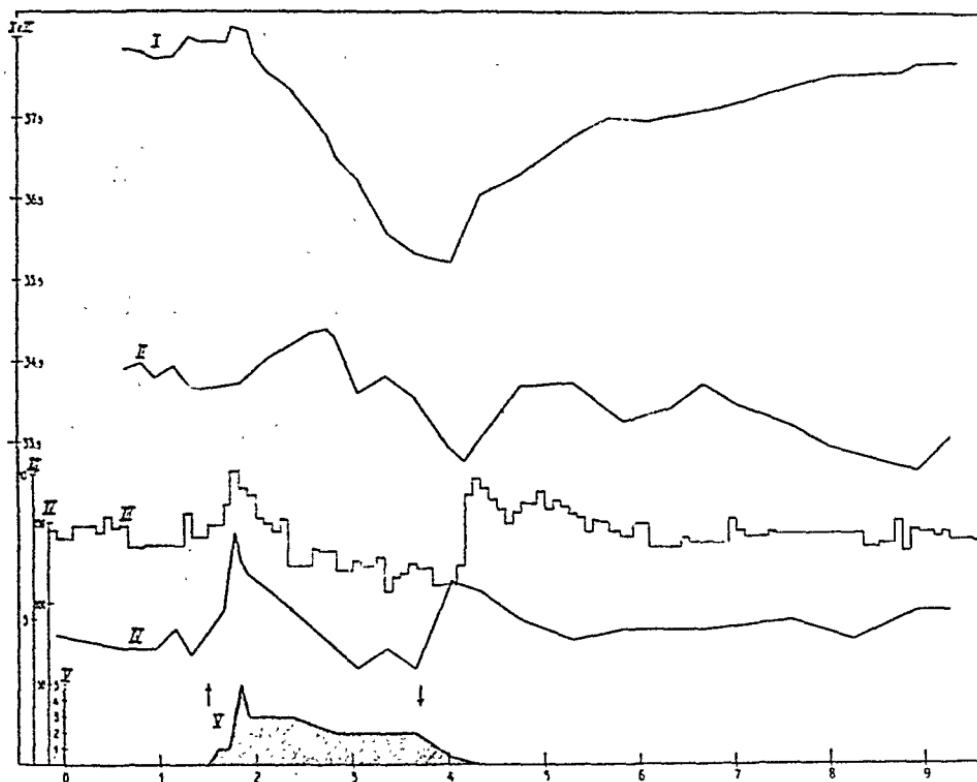


Abb. 10. Versuch vom 15.3.1940. Geschlossenes Respirationssystem mit 87 % Sauerstoff. Gewicht des Versuchstieres 600 gr. Asthma durch 2,5 %ige Histaminlösung hervorgerufen.

Allgemeinzustand des Versuchstieres: Ruhig während des ganzen Versuches.

- I. Rektaltemperatur (Grad Celsius).
- II. Hauttemperatur (Grad Celsius).
- III. Sauerstoffaufnahme in cc pro Minute (reduziert).
- IV. Atemfrequenz pro Minute.
- V. Grad des Asthmas.

Abszisse: Zeit in Stunden.

oder weniger die Herabsetzung der Sauerstoffaufnahme während des Asthmas (Abb. 8, 9, 10 und 13). In anderen Fällen findet man keine deutliche Erhöhung der Sauerstoffaufnahme im ersten Teil der Nachperiode (Abb. 11).

Das unterschiedliche Verhalten der Sauerstoffaufnahme in der

Nachperiode beruht auf einer Differenz zwischen den verschiedenen Versuchen, die sich vor allem in drei Richtungen bewegt: Zum ersten beruht der Sauerstoffverbrauch in der Nachperiode darauf, ob während des Asthma-Anfalles eine Sauerstoffschuld entstanden ist, und wenn dies der Fall war, auf ihrer Grösse; denn diese Sauerstoffschuld muss in der Nachperiode zurück erstattet werden. Ferner kann die Sauerstoffaufnahme im ersten Teil der Nachperiode dadurch etwas beeinflusst werden, dass das Blut während des Asthma-Anfalles nicht mit Sauerstoff gesättigt war. In diesem Fall wird ein Teil des im Anfang der Nachperiode aufgenommenen Sauerstoffes dazu verbraucht, das Blut wieder mit Sauerstoff zu sättigen. Ein weiterer Faktor von Bedeutung ist das Verhalten der Körpertemperatur in der Nachperiode. Ist nämlich die Rektaltemperatur im Beginn der Nachperiode gesenkt und steigt während letzterer an, so nimmt infolgedessen auch die Sauerstoffaufnahme zu; denn zur Erhöhung der Körpertemperatur ist eine Vermehrung der Kalorienproduktion notwendig, vorausgesetzt, dass die Wärmeabgabe unverändert bleibt.

Der in Abb. 9 dargestellte Versuch gibt eine Vorstellung davon, wie diese verschiedenen Faktoren die Sauerstoffaufnahme in der Nachperiode beeinflussen können. In diesem Versuch steigt die Sauerstoffaufnahme nach dem schweren Asthma in wenigen Minuten von  $-75\%$  auf  $+25\%$ . Während dieser Zeit verändert sich weder die Körpertemperatur noch die Hauttemperatur des Versuchstieres. Man dürfte daher damit rechnen, dass die Zunahme der Sauerstoffaufnahme teils der Sättigung des vorher bestimmt nicht mit Sauerstoff gesättigten Blutes, teils der beginnenden Wiedererstattung der Sauerstoffschuld diente. Nimmt man an, dass die Sauerstoff-Sättigung des arteriellen Blutes bei etwa  $50\%$  lag, was man auf Grund des in Abb. 17 wiedergegebenen Versuches wohl mit Recht tun kann, ohne in Gefahr zu sein, sich allzu weit von den tatsächlichen Verhältnissen zu entfernen, so würden 5 cc Sauerstoff notwendig sein, um das Blut wieder vollkommen zu sättigen. Dabei wurde die Blutmenge des Tieres auf 50 cc geschätzt, und der Sauerstoffgehalt bei vollständiger

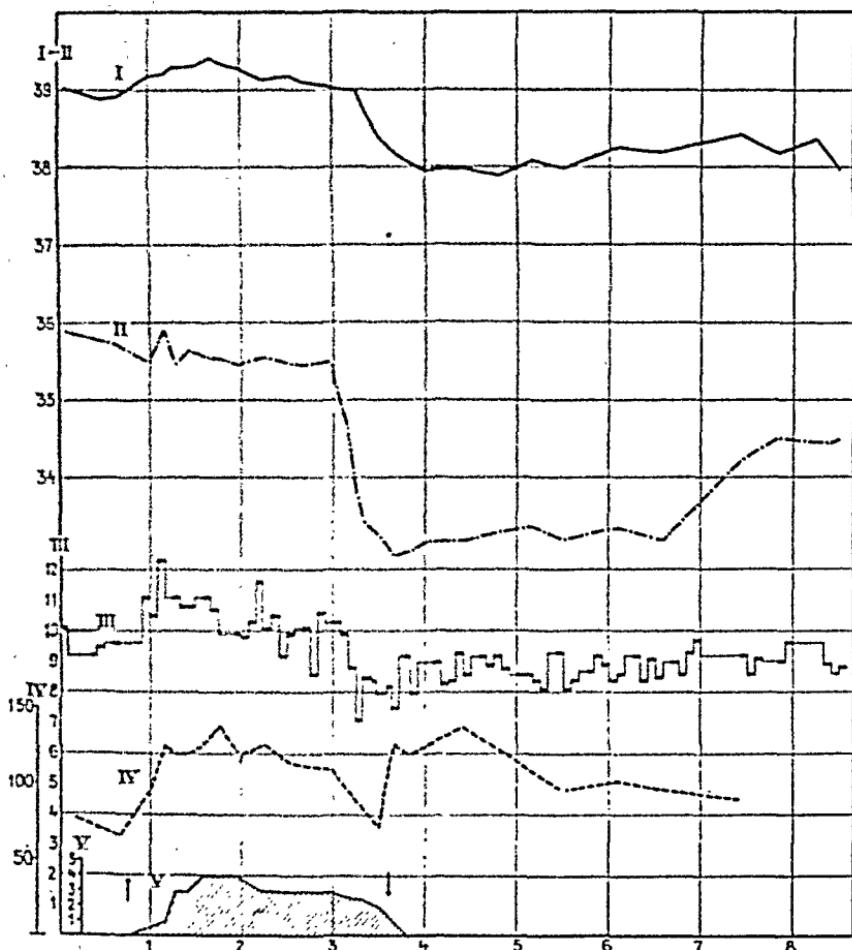


Abb. 11. Versuch vom 23.3.1940. Geschlossenes Respirationssystem mit ca. 90 % Sauerstoff. Gleiches Versuchstier wie in Abb. 10. Gewicht des Tieres 600 gr. Asthma durch etwa 2,5 %ige Histaminlösung hervorgerufen. Allgemeinzustand des Versuchstieres: Das Tier sass während des Versuches bis zur Zeit 3 Std. 15 Min. ruhig, dann legte es sich auf die Seite und sah matt aus. Bis zur Zeit 8 Std. lag es schlapp auf der Seite, danach wurde es wieder frischer und versuchte, sich aufzurichten.

- I. Rektaltemperatur in Grad Celsius.
- II. Hauttemperatur in Grad Celsius.
- III. Sauerstoffaufnahme in cc pro Minute (reduziert).
- IV. Atemfrequenz pro Minute.
- V. Grad des Asthmas.

Abszisse: Zeit in Stunden.

Sättigung auf 20 Volumen-Prozent. In der ersten 10-Minuten-Periode wurde etwa 20 cc mehr Sauerstoff aufgenommen als unter Standardbedingungen. Die übrigen 15 cc Sauerstoff könnten dazu benutzt werden, mit der Wiedererstattung der Sauerstoffschuld zu beginnen, d. h. dass dieser Sauerstoff u. a. zur Resynthese eines Teiles der Milchsäure diente, die während der Anoxie gebildet wurde. Abb. 17 zeigt die Vermehrung der Milchsäure bei einem Versuch.

*Das Verhalten der Temperatur während und nach dem Asthma.*

Bei leichtem Asthma steigt im allgemeinen sowohl die Rektal-, wie die Hauttemperatur an, aber meist nicht mehr als etwa 0,5 Grad. In der Nachperiode sinken die Temperaturen wieder zur Norm.

Bei schwerem Asthma sinkt die Rektal- und die Hauttemperatur um mehrere Grade. Die stärkste Herabsetzung der Temperatur betrug in meinen Versuchen etwa 3 Grad. Die Stärke des Temperaturfalles während des Asthmas beruht teils auf dem Schweregrad des Anfallen, teils auf seiner Dauer. Die stärksten Temperatursenkungen wurden bei langdauerndem Asthma gefunden. In der Nachperiode kehren im allgemeinen die Temperaturen sicherer zum Ausgangswert zurück, wenn das Asthma von kurzer Dauer war, als wenn es lange anhielt. Nach einem kurzdauernden Asthma steigen die Rektal- wie die Hauttemperatur ungefähr gleich rasch an, und erreichen allmählich wieder den Ausgangswert (Abb. 9). Nach einem schwereren und länger dauernden Asthma bleibt die Hauttemperatur oft länger erniedrigt als die Rektaltemperatur (Abb. 10). In manchen Fällen findet man, dass weder die Rektal- noch die Hauttemperatur zu den Normalwerten zurückkehren, auch wenn die Nachperiode auf 5 Stunden ausgedehnt wird. Besonders bemerkenswert ist es, dass der Temperaturanstieg in der Nachperiode immer langsamer vor sich geht, als die Temperatursenkung während des Asthma-Anfallen selbst. Dies Verhalten kann möglicherweise dadurch erklärt werden, dass ein Teil der produzierten Energie für Resynthesen verschiedener Art angewendet wird.

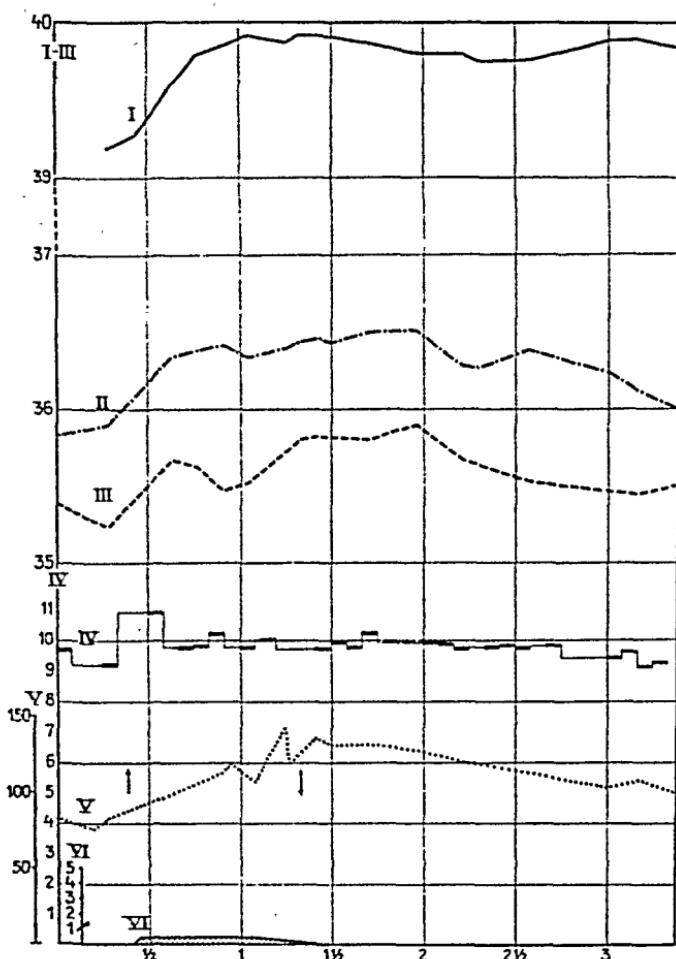


Abb. 12. Versuch vom 17. 2. 1943. Geschlossenes Respirationssystem mit atmosphärischer Luft. Gewicht des Versuchstieres 520 gr. Asthma durch unverdünntes Homoserum ausgelöst (das Tier wurde im Dezember 1942 sensibilisiert).

Allgemeinzustand des Versuchstieres: Ruhig während des ganzen Versuches.

- I. Rektaltemperatur in Grad Celsius.
- II. und III. Hauttemperaturen in Grad Celsius.
- IV. Sauerstoffaufnahme in cc pro Minute (reduziert).
- V. Atemfrequenz pro Minute.
- VI. Grad des Asthmas.

Abszisse: Zeit in Stunden.

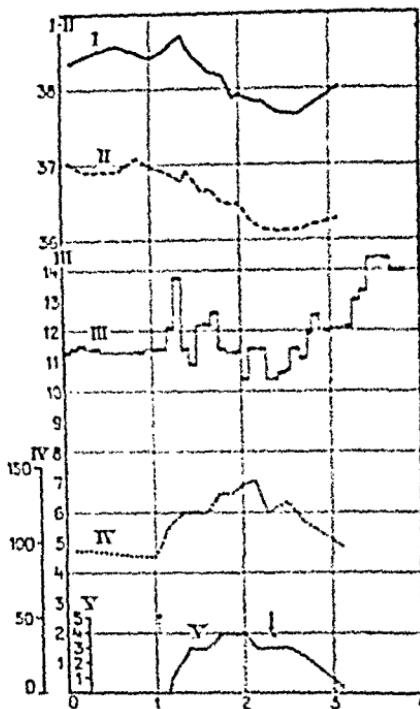


Abb. 13. Versuch vom 30.4.1940. Geschlossenes Respirationssystem mit etwa 50 % Sauerstoff im System. Gewicht des Versuchstieres 885 gr. Asthma durch unverdünntes Homoserum ausgelöst (das Tier wurde im Februar 1940 sensibilisiert).

Allgemeinzustand des Versuchstieres: Ruhig während des ganzen Versuches.

- I. Rektaltemperatur in Grad Celsius.
- II. Hauttemperatur in Grad Celsius.
- III. Sauerstoffaufnahme in cc pro Minute (reduziert).
- IV. Atemfrequenz pro Minute.
- V. Grad des Asthmas.

Abszisse: Zeit in Stunden.

*Die Beziehungen zwischen Temperaturveränderungen und Sauerstoffaufnahme im Asthma. Die Wärmecabgabe im Asthma.*

Es besteht ein intimer Zusammenhang zwischen dem Schweregrad des Asthmas, der Sauerstoffaufnahme und den Veränderungen der Rektal- und Hauttemperatur. So steigt bei leichtem Asthma die Sauerstoffaufnahme, und die Temperaturen des

Versuchstieres erhöhen sich (Abb. 12). Bei schwerem Asthma sinken der Sauerstoffverbrauch wie die Rektal- und Hauttemperatur des Tieres (Abb. 9, 10, 11 und 13). Es ist bemerkenswert, dass die Rektaltemperatur sinken kann, noch bevor die Sauerstoffaufnahme unter den Normalwert herunter gegangen ist (Abb. 10, Zeit 2 Std. 15 Min.). Während des Asthmas gibt es zwar eine enorme Atmungsarbeit, aber da keine äussere mechanische Arbeit vom Organismus geleistet wird, so muss unter diesen Umständen die gesamte Kalorienproduktion gleich der totalen Wärmeproduktion sein. In diesem Fall kann daher *nicht eine verminderte Wärmeproduktion die Ursache der Temperatursenkung sein; sondern letztere muss auf einer erhöhten Wärmeabgabe beruhen*, falls nicht die anaerobe Kalorienproduktion herabgesetzt sein sollte. Dies anzunehmen, haben wir aber keinerlei Veranlassung. Vielmehr dürfte letztere bedeutend erhöht sein. Im Asthma steigt nämlich der Milchsäuregehalt des Organismus (siehe Kapitel V und Kapitel VI, Abschn. 3). In dem in Abb. 10 dargestellten Versuch war in dem betreffenden Zeitpunkt die Hauttemperatur um einige Zehntel Grade erhöht. Dies trug zu der vermehrten Wärme-Abgabe bei. Hierzu kommt noch ein anderer Faktor, der bestimmt eine grosse Bedeutung für die Wärme-Abgabe im Asthma haben dürfte, nämlich die Vermehrung der Bewegungen vor allem des Thorax, aber auch anderer Körperteile. Durch die vermehrten Atembewegungen wird die Wärme-Abgabe durch Konvektion erhöht. Hierüber schreibt Du Bois folgendes (1936; Seite 60): »Small currents of air may increase convection markedly so that there is a deviation from the rate of cooling calculated from Newton's law. Many of our calorimeter experiments have shown that when a man is moving a little or when the air is stirred by a fan he can lose increasing amounts of heat through his skin without a rise in surface temperature and even with a distinct fall. This explains the experiments and theories that had been adversely criticised by physiologists who have forgotten the role of convection.« Die Lungenventilation pro Minute ist bei schwererem Asthma vermindert. Eine Vermehrung der Wärme-Abgabe kann daher auf diesem Wege nicht zu Stande kommen.

Dass die Wärme-Abgabe bei dem in Abb. 10 angeführten Fall vermehrt ist, dürfte auch aus der folgenden Überschlagsberechnung hervorgehen. Ich möchte eine derartige Berechnung nur mit einer gewissen Zurückhaltung anführen; denn es ist nicht möglich, die Faktoren vollkommen zu überblicken, die auf eine solche Berechnung einwirken könnten. Sie kann außerdem notwendigerweise nur approximativ sein, da in sie verschiedene Faktoren eingehen, deren exakte Größe wir im gegebenen Fall nicht genau kennen. So muss beispielsweise in diesen Berechnungen die Rektaltemperatur die Körpertemperatur representieren, obgleich wir wissen, dass letztere in den verschiedenen Teilen des Körpers different ist. Ferner müssen wir mit einem kalorischen Wert für den Sauerstoff und einer spezifischen Wärme des Körpers rechnen, die im einzelnen Fall von den hier eingesetzten Werten de facto abweichen könnten. Bei diesen Berechnungen wurde auch keine Rücksicht auf solche Veränderungen des Energie-Verbrauches im Organismus genommen, die durch osmotische Wirkungen (z. B. eine Veränderung der Urinproduktion) entstehen könnten. Man hat aber kaum eine begründete Veranlassung, zu vermuten, dass in der letztgenannten Beziehung während des Asthmas wesentliche Veränderungen zu stande kommen. Bei diesen Überschlagsberechnungen wurden im Verlauf ihrer Durchführung mehr Zahlen eingesetzt, als unbedingt signifikant sind; denn wenn man in einem frühen Stadium der Berechnung die Werte zu approximativ nimmt, ruft man Gefahr, die möglichen Fehler zu vergrößern.

Zwischen den Zeiten 2.<sup>10</sup> und 4.<sup>20</sup> in dem in Abb. 10 dargestellten Versuch sank die Rektaltemperatur des Versuchstieres von 37,9 auf 35,7°, d. h. um 2,2 Grad. Für diese Periode dürfte die Mitteltemperatur des Tieres auf 36,8° geschätzt werden. (Im allgemeinen wurde als Mitteltemperatur das arithmetische Mittel aus der im Beginn und am Schluss der Periode gemessenen Temperatur angesetzt. Dies dürfte in einer derartigen Überschlagsberechnung als zulässig angesehen werden. Ein Fehler von 0,1° in der berechneten Mitteltemperatur würde für die betreffende Periode in dem berechneten Sauerstoffsverbrauch unter Standardbedingungen einen Fehler von 0,63 % verursachen.) In der Vorperiode war die Temperatur des Versuchstieres 38,2°, gleichzeitig betrug sein Sauerstoffsverbrauch 7,9 cc pro Minute. Nach HEMMINGSEN (1933) geht bei der Ratte eine Veränderung der Körpertemperatur um 1° mit einer Veränderung des Grund-Umsatzes um 6,5 % einher. Diese Zahlen stimmen mit den Werten überein, die KROGH (1916, Seite 143) am Hund beobachtete (5 bis 6 %). Man dürfte daher berechtigt sein, anzunehmen, dass die entsprechenden Werte für Meerschweinchen in der gleichen Größenordnung liegen. Tun wir dies, so müsste im vorliegenden Fall die Sauerstoffaufnahme unter Standardbedingungen für die Zeitperiode 2.<sup>10</sup> bis 4.<sup>20</sup> sein:

$$7,9 - \frac{7,9 \cdot 6,5 \cdot 1,4}{100} = 7,18 \text{ cc pro Minute. Der Sauerstoffsverbrauch für die ganze Periode müsste demnach } 7,2 \cdot 101 = 725 \text{ cc betragen. In Wirklichkeit}$$

wurden während dieser Zeit 692 cc aufgenommen. Demnach wurde in dieser Periode 33 cc weniger Sauerstoff aufgenommen, als das Versuchstier unter Standardbedingungen hätte aufnehmen sollen. Setzt man den kalorischen Wert des Sauerstoffs mit 4,85 an, so entsprechen 33 cc Sauerstoff 160 cal. In der betreffenden Periode besteht demnach in der aeroben Kalorienproduktion ein Defizit von 160 cal. Um die Körpertemperatur des Versuchstieres um  $1^{\circ}$  zu erhöhen, ist eine Wärmemenge von  $600 \cdot 0,83 \cdot 1,0 = 498$  cal. notwendig, wenn man die spezifische Wärme des Körpers mit 0,83 ansetzt. Da im vorliegenden Fall ein Defizit von 160 cal. besteht, so müsste die Körpertemperatur dementsprechend um  $0,32^{\circ}$  sinken, dies unter der Voraussetzung, dass die Wärme-Abgabe den Standardbedingungen entsprach und der Mangel in der aeroben Kalorienproduktion nicht mehr oder weniger durch eine vermehrte anaerobe Kalorienproduktion kompensiert wurde. Wie aber schon früher angeführt, gibt es experimentelle Befunde, die dafür sprechen, dass wir unter diesen Bedingungen eine erhöhte anaerobe Kalorienproduktion haben. Ferner wird während des Asthmas ein Teil des an das Hämoglobin gebundenen Sauerstoffs verbraucht. Unter Berücksichtigung dieser beiden Fakta und weiterhin unter der Voraussetzung, dass die Wärme-Abgabe unverändert blieb, hätte die Rektal-Temperatur um weniger als  $0,32^{\circ}$  sinken müssen. Tatsächlich nahm aber stattdessen die Körpertemperatur um  $2,9^{\circ}$  ab. Dies Verhalten dürfte am besten dadurch erklärt werden, dass während des Asthmas eine vermehrte Wärmeabgabe, wahrscheinlich durch erhöhte Konvektion, erfolgte. Hierdurch könnte das im ersten Augenblick eigentlich wirkende Verhalten erklärt werden, nämlich dass während des Asthmas eine relativ geringe Verminderung der Sauerstoffaufnahme zu einer relativ starken Herabsetzung der Rektaltemperatur führen kann (Abb. 10 und 13).

Ein entsprechendes Verhalten sehen wir in dem in Abb. 9 wiedergegebenen Versuch. Im übrigen unterscheiden sich die beiden genannten Versuche in mehrfacher Hinsicht von einander. Bei dem Versuch in Abb. 9 handelt es sich um ein sehr schweres, aber kurzdauerndes Asthma in atmosphärischer Luft, bei dem Versuch in Abb. 10 um ein weniger schweres, aber länger dauerndes Asthma in einer Atmosphäre mit erhöhtem Sauerstoff-Gehalt. Führen wir auch hier in gleicher Weise, wie für den Versuch in Abb. 10, eine Überschlagsberechnung durch, so ergibt sich folgendes: Der Sauerstoffverbrauch des Versuchstieres ist in der Vorperiode 7,5 cc pro Minute, die Körpertemperatur gleichzeitig etwa  $37,7^{\circ}$ . In der Zeit 2 Std. 30 Min. bis 2 Std. 48 Min. sank die Rektaltemperatur von  $37,60^{\circ}$  auf  $36,76^{\circ}$ . Als Mitteltemperatur für diese Zeit ergibt sich etwa  $37,18^{\circ}$ . Unter Standardbedingungen müsste daher der Sauerstoffverbrauch

des Versuchstieres in dieser Zeitperiode betragen:  $7,5 - \frac{7,5 \cdot 6,5 \cdot 0,52}{100} =$

$7,25$  cc, oder etwa 131 cc für 18 Minuten. In Wirklichkeit nimmt aber das Versuchstier in dieser Zeit nur 77 cc auf. Das Tier hat also in der fraglichen Zeitperiode 54 cc weniger Sauerstoff aufgenommen, als unter

Standardbedingungen. Dies entspricht einem Defizit von 262 Kalorien in der aeroben Kalorienproduktion. Nimmt man ferner in gleicher Weise wie früher an, dass die Wärme-Abgabe der unter Standardbedingungen entsprach, so hätte die Temperatur des Versuchstieres auf Grund der

Verminderung der aeroben Kalorienproduktion um  $\frac{262}{550 \cdot 0,93} = 0,57$  Grad

sinken müssen. Wie aber bereits weiter oben betont, darf man mit Recht annehmen, dass die Herabsetzung der aeroben Kalorienproduktion wenigstens zu einem gewissen Teil durch eine Erhöhung der anaeroben Kalorienproduktion kompensiert wird. Die Temperatur müsste daher um weniger als 0,57 Grad sinken. In Wirklichkeit betrug aber der Temperaturfall 0,81 Grad. Dies dürfte in Übereinstimmung mit dem, was schon früher ausgeführt wurde, wahrscheinlich mindestens zum Teil darauf beruhen, dass die Wärme-Abgabe während des Asthmaes erhöht wurde. Hier ist aber infolge der während der fraglichen Asthmaperiode stark herabgesetzten Sauerstoffaufnahme der Unterschied zwischen der in der genannten Weise berechneten und der tatsächlich gefundenen Senkung der Temperatur bedeutend kleiner als in dem in Abb. 10 dargestellten Versuch.

Ein entsprechendes Verhalten finden wir auch in anderen Versuchen, so beispielsweise in dem in Abb. 19 wiedergegebenen Versuch. Hier sank die Temperatur um 3°, während der aus dem Sauerstoffdefizit berechnete Temperaturfall etwa 1,69° betragen müsste. In manchen Versuchen aber, in denen die Sauerstoffaufnahme während einer langen Zeit stark herabgesetzt war, kann man auch finden, dass die tatsächliche Temperatursenkung kleiner ist, als die berechnete. Dies gilt für den in Abb. 23 wiedergegebenen Versuch. In diesem Versuch sank die Rektaltemperatur um 2,9°, während man auf Grund des Sauerstoffdefizits einen Temperaturfall um etwa 3° hätte erwarten dürfen. Hierzu ist allerdings zu bemerken, dass in diesem Versuch der Sauerstoffverbrauch während der Vorperiode auffallend hoch war; das Versuchstier befand sich also möglicherweise nicht unter Standardbedingungen, was die Beurteilung erschwert. Man müsste in diesem Fall wohl annehmen, dass u.a. eine sehr starke anaerobe Kalorienproduktion dem Absinken der Temperatur entgegenwirkte. (Nach Angaben in der Literatur (siehe NEEDHAM, 1932) geht die Bildung von einem Gramm Milchsäure mit einer totalen Kalorienproduktion von 390 cal. einher.) Ferner muss in diesem Versuch die Wärme-Abgabe durch Konvektion bedeutend kleiner gewesen sein, als in dem in Abb. 10 wiedergegebenen Versuch. Denn in dem Versuch der Abb. 23 war die Hauttemperatur während des grössten Teiles des Asthma-Anfalles gesenkt, während sie in dem Versuch von Abb. 10 während des Asthmaes grösstenteils erhöht war.

In der Nachperiode nach einem schwereren Asthma, während dessen die Temperatur gesenkt war, finden man eine Temperatursteigerung. Bereits früher wurde betont, dass im allgemeinen die Temperaturerhöhung

pro Zeiteinheit durchgehend geringer ist, als die Herabsetzung der Temperatur während des Asthmas. Wenn man eine Überschlagsberechnung ähnlicher Art durchführt, wie für die Verhältnisse während des Asthmas, so findet man, dass die Temperaturerhöhung in der Nachperiode in der Regel geringer ist, als man nach der gemessenen Sauerstoffaufnahme erwarten sollte. Es soll hier nur ein Beispiel angeführt werden, das dieses Verhalten beleuchtet.

Es muss aber klar ausgesprochen werden, dass es schwieriger als für die Verhältnisse während des Asthmaanfalles selbst ist, einigermassen sichere Schlüsse aus einer Überschlagsberechnung über die Verhältnisse in der Nachperiode nach einem schwereren Asthma zu ziehen. Dies geht aus Folgendem hervor: In der Regel sinkt, wie oben erwähnt, die Rektaltemperatur im Asthma stärker, als man nach der Herabsetzung der aeroben Kalorienproduktion erwarten würde. Berechnet man in der oben angegebenen Weise die Differenz zwischen der tatsächlich gefundenen und der erwarteten Temperatursenkung, so muss man sagen, dass diese Differenz zu klein ist; denn der Herabsetzung der aeroben Kalorienproduktion, die der Berechnung zu Grunde liegt, wirkt eine Steigerung der anaeroben Kalorienproduktion mehr oder weniger entgegen. In der Nachperiode aber steigt in der Regel die Rektaltemperatur weniger, als man nach der Erhöhung der aeroben Kalorienproduktion erwarten sollte. Der Unterschied zwischen der berechneten und der gefundenen Temperatursteigerung ist zu gross; denn ein Teil der Energie wurde durch die Resynthese von Glykogen gebunden, und konnte daher nicht Wärme bilden.

Bei der Analyse dieser Verhältnisse in den Versuchen, die in Abb. 9 wiedergegeben sind, ist es am zweckmässigsten, die erste Nachperiode nach dem Asthma-Anfall in mehrere kleinere Perioden aufzuteilen. Auf diese Weise lassen sich die Verhältnisse in Bezug auf die Wärme-Abgabe leichter beurteilen. Als erste Periode wählen wir die Zeit von 2 Std. 50 Min. bis 3 Std. 30 Min. Während dieser Zeit ist die Atemfrequenz erhöht, und mit Rücksicht auf die Resultate, die im nächsten Kapitel besprochen werden, können wir mit Recht vermuten, dass auch die Lungenventilation erhöht ist. Beide Veränderungen bewirken eine Vermehrung der Wärme-Abgabe. Was die Frequenzerhöhung betrifft, so ruft diese durch vermehrte Konvektion eine Steigerung der Wärme-Abgabe hervor. Wir dürfen aber voraussetzen, dass diese durch erhöhte Konvektion bewirkte Vergrösserung der Wärme-Abgabe hinter der während des Asthmas zurückbleibt. Die Atemzüge sind jetzt in der Nachperiode zwar beschleunigt, aber klein und gleichmässig, zum Unterschied von den vertieften und erschwerten Atemzügen während des Asthma-Anfalles. Was die Steigerung der Lungenventilation betrifft, so können wir unter Hinweis auf die Versuchsergebnisse des nächsten Kapitels annehmen, dass bei Tieren von der Grösse, wie sie im allgemeinen für Respirationsversuche angewendet werden, die Mehrventilation in der ersten Nach-

periode etwa 100 cc pro Minute beträgt. Die Erhöhung der Wärme-Abgabe, die hierdurch zu Stande kommt, kann infolgedessen für die herrschenden Versuchsbedingungen ungefähr geschätzt werden. Im Respirations-Apparat ist die Luft bei etwa 32° Temperatur praktisch genommen mit Feuchtigkeit gesättigt. Das Versuchstier atmet eine mit Feuchtigkeit gesättigte Luft von etwa 38° aus. Die Wassermenge, die auf diese Weise das Tier in Form von Wasserdampf verlässt, beträgt etwa 0,013 gr pro Liter. Dies entspricht einem Wärmeverlust durch Wasserabduktion in Höhe von  $0,013 \cdot 586 = \text{ca. } 8 \text{ cal. pro Liter Mehrventilation}$ . Ausserdem verliert das Tier Wärme durch die Erwärmung der vermehrten Ausatmungsluft und durch die gesteigerte Kohlensäure-Ausscheidung im Beginn der Nachperiode. Diese beiden Faktoren sind aber vergleichsweise klein, im Verhältnis zu den Wärmemengen, die den Organismus durch Wasserabduktion verlassen. In dieser Teilperiode dürfte demnach die Wärme-Abgabe vermehrt sein, verglichen mit den Verhältnissen unter Standardbedingungen. Als nächste Teilperiode der Nachperiode wählen wir die Zeit von 3 Std. 30 Min. bis 4 Std. 10 Min. In dieser Periode ist die Atemfrequenz normal. Wir dürfen daher annehmen, dass die Wärme-Abgabe der unter Standardbedingungen entspricht.

In der ersten Teilperiode, nämlich in der Zeit von 2 Std. 50 Min. bis 3 Std. 30 Min., stieg die Körpertemperatur von 36,75° auf 37,16°. Die Mitteltemperatur für diese Periode darf daher mit 36,96° angesetzt werden. Der Sauerstoffverbrauch unter basalen Bedingungen hätte demnach in

dieser Periode betragen sollen:  $7,5 - \frac{7,5 \cdot 6,5 \cdot 0,74}{100} = 7,14 \text{ cc pro Minute}$ ,

also während 40 Minuten 285,6 cc. In Wirklichkeit nahm das Versuchstier während dieser Zeit 342,5 cc Sauerstoff auf. Es atmete also 56,9 cc mehr ein, als es unter Standardbedingungen hätte tun sollen. Dies entspricht einer Kalorienmenge von  $56,9 \cdot 4,85 = 276 \text{ cal}$ . Während der betreffenden Periode stieg die Temperatur um 0,41 Grad. Hierfür wurden gebraucht:  $550 \cdot 0,41 \cdot 0,83 = 187 \text{ cal}$ . Der Überschuss beträgt demnach etwa 90 cal. Diese 90 cal. dürften dazu benutzt werden, u. a. die Milchsäure zu resynthetisieren, die während des vorhergehenden anoxaemischen Zustandes gebildet wurde, das ungesättigte Blut wieder mit Sauerstoff zu sättigen und vermehrt Wärme abzugeben. Es ist bemerkenswert, dass demnach nur ein Teil dieser 90 cal. zur Resynthese der Milchsäure verwendet werden kann. Damit nicht die totale Kalorienproduktion während des Asthma in dem betreffenden Versuch sinken sollte, wäre eine anaerobe Kalorienproduktion von etwa 260 cal. erforderlich. Diese Diskrepanz könnte möglicherweise dafür sprechen, dass in dem betreffenden Versuch die totale Kalorienproduktion während des Asthma erniedrigt war.

Während der zweiten Teilperiode, nämlich von 3 Std. 30 Min. bis 4 Std. 10 Min., stieg die Körpertemperatur von 37,16° auf 37,32°. Als Mitteltemperatur können daher für diese Periode 37,24 Grad angesetzt werden. Der Sauerstoffverbrauch müsste in dieser Periode unter basalen

Bedingungen 7,28 cc pro Minute oder 291 cc während der 40 Minuten betragen. In Wirklichkeit wurden in dieser Zeit 311,6 cc Sauerstoff aufgenommen. Das Versuchstier hatte also 20,6 cc mehr eingearmet als unter Standardbedingungen. Dies entspricht etwa 110 cal. Die Temperatur stieg während dieser Zeit um 0,16 Grad. Hierfür sind etwa 73 cal. erforderlich. Für die Rückerstattung der Sauerstoffschuld steht also nur ein Überschuss von ca. 27 cal. zur Verfügung. Dieser Wert ist aber so klein, dass er innerhalb der Fehlertoleranzen der Methodik liegt. Es ist daher möglich, dass die gesamte Steigerung der Sauerstoffaufnahme über die Standardwerte hinaus in dieser Periode für die Erhöhung der Körpertemperatur angewendet wurde. Die Sauerstoffschuld müsste in diesem Fall bereits während der vorhergehenden Periode vollkommen zurückgestattet sein.

Aus den obigen Berechnungen können folgende Schlüsse gezogen werden: 1.) Bei schwererem Asthma sinkt die Körpertemperatur häufig mehr, als es der Verminderung der Sauerstoffaufnahme entsprechen würde. Dies kann vermutlich am besten so erklärt werden, dass die Wärmeabgabe vor allem durch vermehrte Konvektion erhöht ist. 2.) Der grösste Teil des Sauerstoffverbrauches in der Nachperiode wird wahrscheinlich für eine Erhöhung der Körpertemperatur angewendet.

Bevor die in diesem Kapitel gewonnenen Versuchsergebnisse zusammengefasst werden, ist es notwendig, kurz einige der Angaben zu berühren, die in der Literatur über Veränderungen der Sauerstoffaufnahme und der Körpertemperatur nach der Injektion von Histamin vorliegen. Ferner ist es nötig, kurz auf die Angaben einzugehen, die die Einwirkung der Kohlensäure auf die Sauerstoffaufnahme betreffen. Denn bei schwerem Asthma müssen wir mit einer Erhöhung der Kohlensäuretension im Organismus rechnen (siehe Kapitel VI, Abschn. 2).

Nach ABELIN (1922) hat Histamin, Ratten von etwa 125 gr Gewicht subcutan injiziert, bis zu Dosen von 20 mg keinen sicheren Effekt auf den Gaswechsel. KNIPPING und STEIGER (1929) zeigten, dass kleine Dosen von Histamin (1 bis 3 mg) beim Hund keine Veränderung des Sauerstoffverbrauches bewirken. Grössere Dosen (15 bis 30 mg) erhöhen die Sauerstoffaufnahme erheblich. EPPINGER, LAZLO und SCHÜRMAYER (1928) zeigten an narkotisierten Hunden, dass der Sauerstoffverbrauch in der ersten Zeit nach der Injektion von Histamin, gleichzeitig mit der Blutdrucksenkung, erniedrigt wurde. Mit dem Verschwinden der Blutdrucksenkung wurde die Sauerstoffaufnahme erheblich über die Norm hinaus erhöht. Die Verfasser nehmen an, dass die Veränderungen im Sauerstoffverbrauch durch die Zirkulations-Veränderungen verursacht werden, und dass sie

nicht auf einer spezifischen Wirkung des Histamins auf den Stoffwechsel beruhen. Beim Menschen wurde in der Regel nach der Injektion von Histamin eine unbedeutende Erhöhung des respiratorischen Gaswechsels gefunden (u. a. v. EULER und LILJESTRAND, 1929).

Bezüglich der Veränderungen der Körpertemperatur nach der Injektion von Histamin stimmen die Literaturangaben nicht vollkommen überein. Die meisten Verfasser berichten von einer Senkung der Körpertemperatur nach grösseren Dosen und von dem Fehlen jeglicher Wirkung nach kleineren Dosen (u. a. LESCHKE, 1913). Es müsste demnach ein Unterschied zwischen kleinen Histamindosen und kleinen Antigenmengen bestehen, indem nur die letzteren eine leichte Temperaturerhöhung bewirken könnten.

In der Literatur liegen verschiedene Angaben darüber vor, dass eine Erhöhung der Kohlensäurespannung in der Einatmungsluft den Sauerstoffverbrauch herabsetzt (u. a. ILTZHÖFER, 1920, GESELL und Mitarbeiter, 1930, REIN, 1936, REIN und OTTO, 1940).

REIN zeigte, dass bei Menschen und Hunden die Einatmung eines Kohlensäure-Luftgemisches, das 0,5 bis 6 % Kohlensäure enthielt, eine Senkung des Gesamtsauerstoffverbrauches bewirkte, die bis zu 20 % des Sauerstoffverbrauches unter Standardbedingungen gehen konnte.

Wie schon früher betont, wurden beim experimentellen Asthma prinzipiell die gleichen Veränderungen der Sauerstoffaufnahme und der Körpertemperatur gefunden, gleichgültig ob das Asthma durch Histamin oder auf allergischem Wege ausgelöst wurde. Ferner zeigte sich immer, dass zwischen dem Grad der Atmungsbehinderung und der Grösse der Sauerstoffaufnahme ein intimer Zusammenhang besteht. Sobald die Atmungsschwerung aufhört, erhöht sich beinahe momentan die Sauerstoffaufnahme (wie in Abb. 3). Man dürfte daher mit Recht annehmen, dass die gefundenen Veränderungen der Sauerstoffaufnahme (und damit auch die der Körpertemperatur, denn letztere zeigt einen intimen Zusammenhang mit der Sauerstoffaufnahme) die Folge der Atmungsbehinderung sind, und nicht durch resorpitive Wirkung der Asthma-auslösenden Stoffe verursacht werden. Nach den oben angeführten Angaben der Literatur, betreffend die Einwirkung von Histamin auf den Gaswechsel, könnte man sich aber möglicherweise denken, dass die Erhöhung der Sauerstoffaufnahme, wie sie bei leichtem Asthma vorkommt, zum Teil durch die steigernde Wirkung verursacht sein könnte, die Histamin auf den Gaswechsel aus-

übt. Andererseits ist es möglich, dass die Erhöhung der Kohlensäurespannung im Organismus während des schweren Asthmas zu der Herabsetzung der Sauerstoffaufnahme beitrug. Die wichtigste Ursache der letzteren dürfte aber die Erschwerung des Gasaustausches in den Lungen sein, da das arterielle Blut im schweren Asthma nicht mit Sauerstoff gesättigt wird, wie aus den Ausführungen in Kapitel V hervorgeht.

### Zusammenfassung.

Die wichtigsten Resultate, die in diesem Kapitel gewonnen wurden, können folgenderweise zusammengefasst werden: *Im leichten experimentellen Asthma des Meerschweinchens steigt die Sauerstoffaufnahme um 10 bis 20 %. Gleichzeitig erhöht sich die Rektal- und Hauttemperatur der Versuchstiere. Bei schwerem Asthma sinken die Sauerstoffaufnahme wie die Rektal- und Hauttemperatur der Versuchstiere erheblich unter die in der Vorperiode gefundenen Werte. Das Ausmass der Verminderung der Sauerstoffaufnahme bei schwerem Asthma ist in hohem Grad abhängig von der Atmosphäre, in der sich das Tier befindet. Die Abnahme ist in einer sauerstoffreichen Atmosphäre bedeutend geringer. In der Nachperiode nach einem leichten Asthma sinken die Sauerstoffaufnahme und die Körpertemperatur wieder auf die Ausgangswerte. In der Nachperiode nach einem schweren Asthma, während dessen die Sauerstoffaufnahme vermindert war, ist dagegen die Sauerstoffaufnahme meist erhöht.* Während der Nachperiode steigt in diesem Fall auch die Rektal- und die Hauttemperatur der Versuchstiere allmählich an, und erreicht in der Regel den Ausgangswert. Die erhöhte Sauerstoffaufnahme in der Nachperiode nach einem schweren Asthma dürfte zur Wiedersättigung des ungenügend gesättigten Blutes, zur Wiedererstattung der Sauerstoffschuld und zu einer Steigerung der Körpertemperatur verwendet werden. Der grösste Teil der Mehraufnahme von Sauerstoff dürfte zur Erhöhung der Körpertemperatur dienen.

Da die Rektaltemperatur meist den Schwankungen der Sauerstoffaufnahme folgt, dürfte man mit Recht annehmen, dass die Veränderungen in der Körpertemperatur der Versuchstiere zum

grössten Teil durch die Veränderungen der Sauerstoffaufnahme bedingt werden.

*Bei leichterem Asthma ist die aerobe Kalorienproduktion etwas erhöht. Bei schwererem Asthma dagegen ist die aerobe Kalorienproduktion vermindert.* Inwieweit auch die totale Kalorienproduktion herabgesetzt ist, lässt sich nicht sicher entscheiden, wenn auch gewisse auf Grund der Versuchsergebnisse durchgeführte Berechnungen möglicherweise hierfür sprechen dürften. Die Entscheidung dieser Frage beruht nämlich darauf, ob die Verminderung der aeroben Kalorienproduktion vollkommen durch eine Erhöhung der anaeroben Kalorienproduktion kompensiert werden kann. (Dies könnte durch direkte Kalorimetrie entschieden werden, mittels deren die totale Wärme-Abgabe des Versuchstieres bestimmt werden könnte. Da die Veränderungen der Körpertemperatur der Versuchstiere bekannt sind, könnte darnach die totale Kalorienproduktion berechnet werden.)

Die Temperatursenkung, die bei schwererem Asthma zu beobachten ist, dürfte mindestens zum Teil durch eine vermehrte Wärme-Abgabe verursacht sein, vermutlich durch erhöhte Konvektion. Dieser wird aber durch die in der Regel gleichzeitig gesenkte Hauttemperatur mehr oder weniger entgegengewirkt. Inwieweit die Senkung der Körpertemperatur bei einem Teil der Fälle auch auf einer Herabsetzung der totalen Wärmeproduktion beruhen kann, lässt sich auf Grund des vorliegenden Materials nicht sicher entscheiden.

## KAP. V.

### Versuche mit der Douglas-Methode.

Die Versuche in diesem Kapitel wurden vor allem in der Absicht durchgeführt, die Veränderungen der Lungenventilation und des respiratorischen Quotienten zu untersuchen, die während des Asthmas auftreten. Auch hier konnte kein prinzipieller Unterschied zwischen dem Histamin-Asthma und dem anaphylaktischen Asthma konstatiert werden.

#### 1. Versuche ohne Narkose.

Die Ausführung dieser Versuche war mit gewissen Schwierigkeiten verknüpft, da die Versuchstiere, wahrscheinlich wegen der Zwangshaltung mit fixiertem Kopf, die Behinderung der Atmung und den Sauerstoffmangel schlecht vertragen. Die Versuche lassen sich besser durchführen, wenn die Tiere ein sauerstoffreicheres Luftgemisch einatmen. Aber selbst dann vertragen sie kein so schweres Asthma, wie im geschlossenen System. In gewöhnlicher atmosphärischer Luft halten sie nur ein mittelschweres Asthma aus. Ein prinzipieller Unterschied zwischen der Stenoseatmung in atmosphärischer Luft und der in einer sauerstoffreicherem Atmosphäre ist im übrigen nicht festzustellen. Das gilt ebenso für das durch Histamin wie für das auf allergischem Wege ausgelöste Asthma.

*Die Atemfrequenz.* In der Regel steigt die Atemfrequenz an, sobald das Asthma einsetzt. Im weiteren Verlaufe des Asthmas verhält sich die Atemfrequenz etwas verschieden, im allgemeinen ist aber die Frequenz bei leichterem Asthma stärker erhöht, als bei schwerem Asthma. In diesen Versuchen sinkt die Atemfrequenz während des Asthmas nicht unter die Normalwerte,

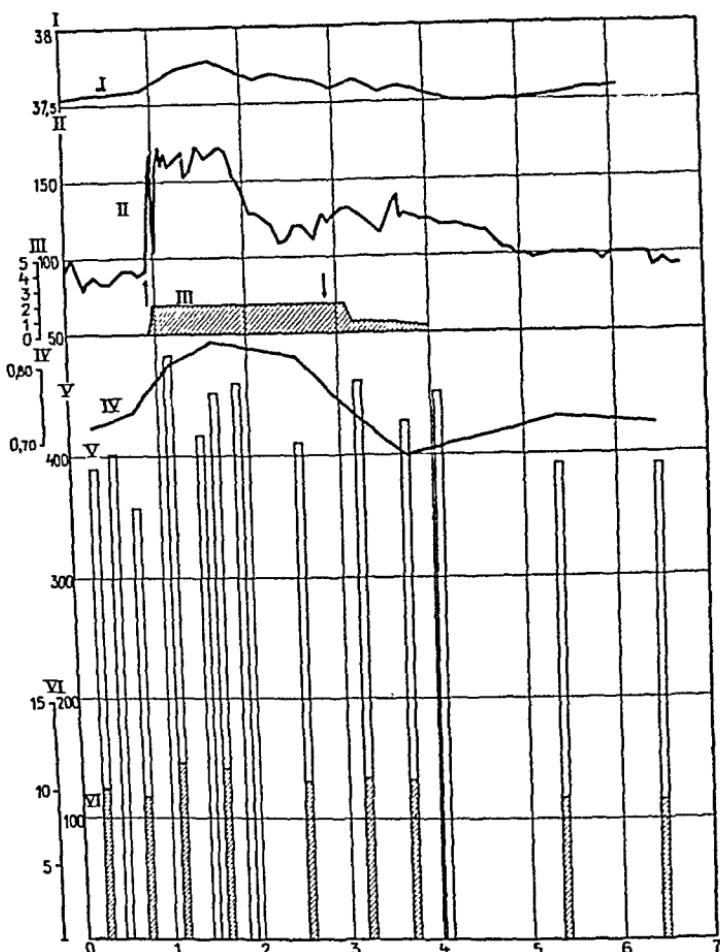


Abb. 14. Versuch vom 18.11.1940. Offenes Respirationssystem. Gewicht des Versuchstieres 760 gr. Das Tier atmete atmosphärische Luft ein. Das Asthma wurde durch unverdünntes Homoserum ausgelöst. Das Versuchstier wurde im April 1940 sensibilisiert.

Allgemeinzustand des Tieres: Ruhig während des ganzen Versuches.

- I. Rektaltemperatur in Grad Celsius.
- II. Atemfrequenz pro Minute.
- III. Grad des Asthmas.
- IV. Respiratorischer Quotient.
- V. Lungenventilation in cc pro Minute (reduziert).
- VI. Sauerstoffaufnahme in cc pro Minute (reduziert).
- Abszisse: Zeit in Stunden.

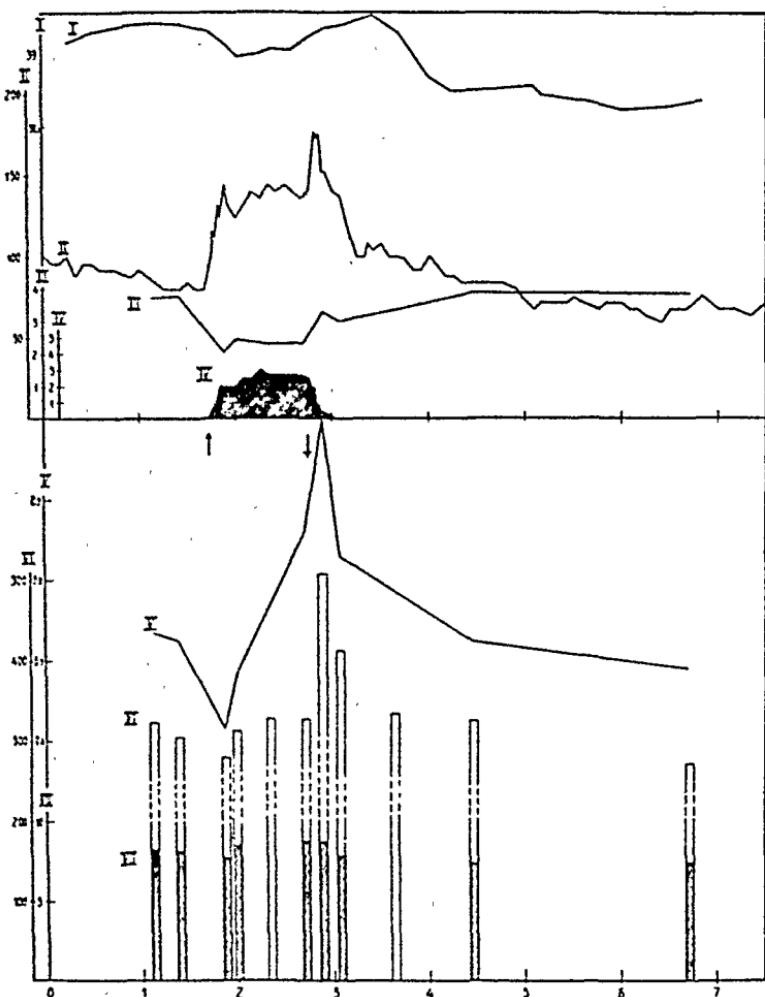


Abb. 15. Versuch vom 29. 11. 1940. Offenes Respirationssystem. Gewicht des Versuchstieres 560 gr. Das Tier atmete atmosphärische Luft ein. Das Asthma wurde durch 0,5 %ige Histaminlösung hervorgerufen.  
Allgemeinzustand des Tieres: Ruhig.

- I. Rektaltemperatur in Grad Celsius.
- II. Atemfrequenz pro Minute.
- III. Atemvolumen (Volumen des einzelnen Atemzuges) in cc (reduziert).
- IV. Grad des Asthmas.
- V. Respiratorischer Quotient.
- VI. Lungenventilation in cc pro Minute (reduziert).
- VII. Sauerstoffverbrauch in cc pro Minute (reduziert).

Abszisse: Zeit in Stunden.

wie es bei denen im geschlossenen System der Fall war. Dies dürfte, wie oben erwähnt, darauf beruhen, dass mit dieser Methodik die schwersten Asthma-Formen nicht untersucht werden konnten. In der Nachperiode nach den leichtesten Asthma-Formen sinkt die Atemfrequenz allmählich wieder zur Norm (siehe Abb. 14). In der Nachperiode nach einem etwas schwereren Asthma steigt die Atemfrequenz meist im gleichen Masse an, wie die Atmung leichter wird. Diese Frequenzerhöhung kommt gut in Abb. 15 zum Ausdruck; wir sehen dort in der Nachperiode eine weitere Steigerung der Atemfrequenz, die sich bereits während des ganzen Asthmas auf einem hohen Niveau gehalten hatte.

*Die Lungenventilation.* Die Lungenventilation pro Minute beträgt bei Meerschweinchen von etwa 600 gr Gewicht ca. 300 cc pro Minute (reduziert). Die Lungenventilation pro Minute wird bei leichterem Asthma grösser (Abb. 14), bei schwererem Asthma kleiner (Abb. 16). Zwischen diesen beiden Extremen gibt es alle Übergänge. So verändert sich in Abb. 15 die Lungenventilation kaum. In der Nachperiode nach einem leichten Asthma sinkt die Lungenventilation wieder zur Norm ab (Abb. 14). In der Nachperiode nach einem schwereren Asthma dagegen finden wir eine erhebliche Erhöhung der Lungenventilation, die erst allmählich wieder zu normalen Werten zurückkehrt (Abb. 15 und 16):

*Die Sauerstoffaufnahme.* Der Sauerstoffverbrauch von Meerschweinchen der Grösse, wie sie in der Regel für die Versuche verwendet wurden (etwa 500 bis 600 gr), beträgt ca. 8 cc pro Minute (reduziert). Ungefähr der gleiche Wert wurde in Kapitel IV mit der geschlossenen Respirationsmethode gefunden.

Die Sauerstoffaufnahme ist bei leichterem Asthma häufig um 10 bis 20 % erhöht (Abb. 14).<sup>1</sup> Bei schwereren Asthma-Formen findet man dagegen eine Senkung der Sauerstoffaufnahme. Dies

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<sup>1</sup> Die Erhöhung der Sauerstoffaufnahme ist demnach bei leichterem Asthma nicht gross. Es bestände daher die Möglichkeit, dass es sich nur um Zufallsbefunde handeln könnte. Man kann die Wahrscheinlichkeit statistisch berechnen, ob die Erhöhung der Werte in einem bestimmten Versuch nur auf einem Zufall beruht. Zu diesem Zweck benutzte ich die folgenden Formeln zur Berechnung des Grades der Signifikanz in der

geht aus Abb. 16 hervor. In diesem Versuch war die Senkung am deutlichsten gegen Ende der Asthmaperiode, während gleichzeitig die Atmungsbehinderung selbst etwas weniger ausgesprochen war, als im Anfang der Periode. Dies muss wahrscheinlich so erklärt werden, dass nicht nur die Stenoseatmung selbst, sondern auch andere Veränderungen in den Lungen den Gasaustausch erschweren. Man sieht häufig, dass der Schweregrad des anaphylaktischen Asthmas im Verlaufe des Versuches abnimmt, obgleich die Konzentration des inhalierten Antigens unverändert blieb. Bei der Inhalation von Histamin kann man ein derartiges Verhalten nicht beobachten.

In der Nachperiode nach einem leichteren Asthma geht die Sauerstoffaufnahme wieder zur Norm herunter (Abb. 14). In der Nachperiode nach einem schweren Asthma findet man dagegen eine deutliche Steigerung der Sauerstoffaufnahme über die für Standardbedingungen geltenden Werte (Abb. 16).

*Der respiratorische Quotient.* Der respiratorische Quotient ist bei Atmung von atmosphärischer Luft etwa 0,70 bis 0,75.

Bei leichterem Asthma steigt der respiratorische Quotient gleichzeitig mit der Erhöhung der Lungenventilation etwas an. In der Nachperiode sinkt der respiratorische Quotient wieder auf seinen Ausgangswert (Abb. 14).

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Differenz der Mittelwerte kleiner Versuchsserien, die FISHER (1936, Seite 128) entnommen ist:

$$\begin{aligned} & x_1 x_2 \dots x_{n_1+1} \quad x'_1 x'_2 \dots x'_{n_2+1} \\ \bar{x} = & \frac{1}{n_1+1} S(x), \quad \bar{x}' = \frac{1}{n_2+1} S(x'), \\ s^2 = & \frac{1}{n_1+n_2} \{ S(x - \bar{x})^2 + S(x' - \bar{x}')^2 \}, \\ t = & \frac{\bar{x} - \bar{x}'}{s} \sqrt{\frac{(n_1+1)(n_2+1)}{n_1+n_2+2}}, \\ n = & n_1 + n_2. \quad n = \text{Zahl der Freiheitsgrade.} \end{aligned}$$

Wenn man auf diese Weise in dem in Abb. 14 wiedergegebenen Versuch den Unterschied in der Sauerstoffaufnahme einerseits während der Asthma-Periode, andererseits während der Vor- und Nachperiode untersucht, findet man einen Wert von  $t = 4,6$ . Aus den Tabellen geht hervor, dass weniger als 1 % Wahrscheinlichkeit dafür besteht, dass die Erhöhung der Sauerstoffaufnahme während der Asthmaperiode nur auf einem Zufall beruhen könnte.

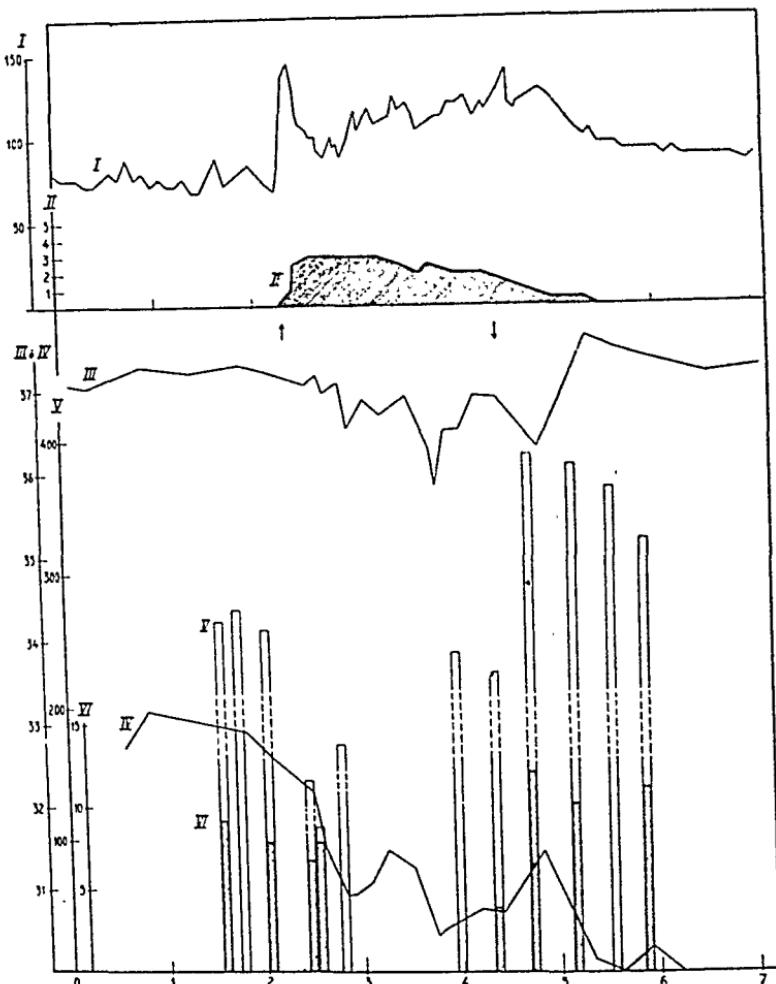


Abb. 16. Versuch vom 18. 12. 1940. Offenes Respirationssystem. Gewicht des Versuchstieres 980 gr. Das Tier atmete 62 % Sauerstoff in Stickstoff ein. Das Asthma wurde durch 5-fach verdünntes Homoserum ausgelöst. Das Tier wurde im April 1940 sensibilisiert.

Allgemeinzustand des Tieres: Ruhig während des ganzen Versuches. Der respiratorische Quotient sinkt von 0,67 vor Beginn des Versuches auf 0,18 und 0,22 in der ersten Zeit des Asthmas und steigt gegen Ende der Asthmaperiode wieder auf 0,63.

- I. Atemfrequenz pro Minute.
- II. Grad des Asthmas.
- III. Rektaltemperatur in Grad Celsius.
- IV. Hauttemperatur in Grad Celsius.
- V. Lungenventilation in cc pro Minute.
- VI. Sauerstoffaufnahme in cc pro Minute (reduziert).

Abszisse: Zeit in Stunden.

Bei schwererem Asthma wird der respiratorische Quotient erheblich kleiner, vor allem wenn das Asthma etwa so schwer ist, dass sich die Lungenvентilation vermindert (Abb. 16. Hier sank der respiratorische Quotient auf etwa 0,20.). Aber auch in den Fällen, in denen sich die Lungenventilation während des Asthmas nicht wesentlich veränderte, so beispielsweise in Abb. 15, findet man ein Absinken des respiratorischen Quotienten im Anfang der Asthmaperiode. Dies kann wahrscheinlich durch die unzulängliche Durchmischung der Atmungsluft bei der Stenoseatmung erklärt werden (vergl. ROELSEN, 1937). Während des Asthmas steigt dann der respiratorische Quotient allmählich an, auch wenn sich die Lungenventilation nicht merkbar verändert. Dies dürfte so gedeutet werden, dass infolge der Erhöhung des Milchsäure-Gehaltes im Organismus mehr Kohlensäure ausgeatmet wird. Im Beginn der Nachperiode kann der respiratorische Quotient noch etwas weiter ansteigen (Abb. 15). Er erreicht dann allmählich wieder den Normalwert, nachdem er eventuell zuerst etwas unterhalb der Norm lag.

Die Veränderungen im respiratorischen Quotienten während des Asthmas dürften demnach teils auf Veränderungen der Lungenventilation, teils auf solchen im Milchsäure-Gehalt des Organismus beruhen. Dagegen konnte unter den vorliegenden Bedingungen nicht entschieden werden, ob nicht auch eine Verschiebung im Verhältnis der verbrannten Eiweiss-, Fett- und Kohlehydratmengen zueinander erfolgte.

*Das Verhalten der Temperatur.* Die Rektal-, wie die Hauttemperatur verhalten sich in prinzipiell gleicher Weise, wie es

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Abb. 16 Forts.

(Die Analysen wurden in diesem Versuch sowohl mit dem van Slyke-Apparat, wie mit dem Haldane-Apparat ausgeführt. Um letzteren anwenden zu können, musste zunächst eine gewisse Menge Stickstoff in den Apparat eingeführt und sauerstofffrei gemacht werden. Erst dann kann die Probe aufgenommen und mit dem Stickstoff gemischt werden, der so lange in der Kohlensäure-Absorptionsbürette aufbewahrt wurde. Hierdurch vermindert sich selbstverständlich die Genauigkeit der Methodik, und wird kleiner, als in Kapitel III angegeben. Die mit der Haldane- und der van Slyke-Methode gefundenen Werte stimmen aber gut überein.)

früher für die Versuche im geschlossenen Respirationssystem in Kapitel IV beschrieben wurde. So steigt die Rektaltemperatur um einige Zehntel Grade bei leichtem Asthma (Abb. 14) und sinkt bei schwererem Asthma (Abb. 16). Auch hier findet man, dass die Rektaltemperatur in der Nachperiode nach einem schweren Asthma rascher wieder auf den Ausgangswert ansteigt, als die Hauttemperatur, die noch längere Zeit erniedrigt bleiben kann (Abb. 16).

## 2. Versuche in Narkose.

In diesen Versuchen wurde ausschliesslich Histamin zur Auslösung des Asthmas angewendet.

Die Versuchstiere ertragen unter diesen Bedingungen auch ein schweres Asthma in atmosphärischer Luft, wahrscheinlich deshalb, weil ihr Sauerstoffverbrauch infolge der Narkose herabgesetzt ist.

*Die Atemfrequenz.* Die Atemfrequenz ist bei leichtem Asthma erhöht und kann bei schwerem Asthma unter den Normalwert sinken.

*Die Lungenventilation.* Die Lungenventilation wird bei leichtem Asthma grösser. Bei schwerem Asthma kann sie erheblich unter den Normalwert heruntergehen. In Abb. 17 zeigt sich mehrmals deutlich, dass die Grösse der Lungenventilation vom Grad des Asthmas abhängt. Wenn der Asthma-Anfall nachzulassen beginnt, wird die Lungenventilation erhöht.

*Der Sauerstoffverbrauch* ist etwa 20 % niedriger als bei Tieren ohne Narkose. Bei leichterem Asthma steigt die Sauerstoffaufnahme etwas an, bei schwererem Asthma kann sie erheblich unter den Normalwert sinken. In der Nachperiode nach einem schweren Asthma findet man hier nicht immer einen kompensatorischen Anstieg. Der respiratorische Quotient ist im allgemeinen etwa 0,80. Im Asthma verhält er sich ebenso, wie bei den Versuchstieren ohne Narkose. Auch die Rektaltemperatur verändert sich in gleicher Weise, wie bei Tieren ohne Narkose. Dies ist gut aus dem in Abb. 17 wiedergegebenen Versuch ersichtlich. Bevor in diesem Versuch das Asthma einen höheren Grad

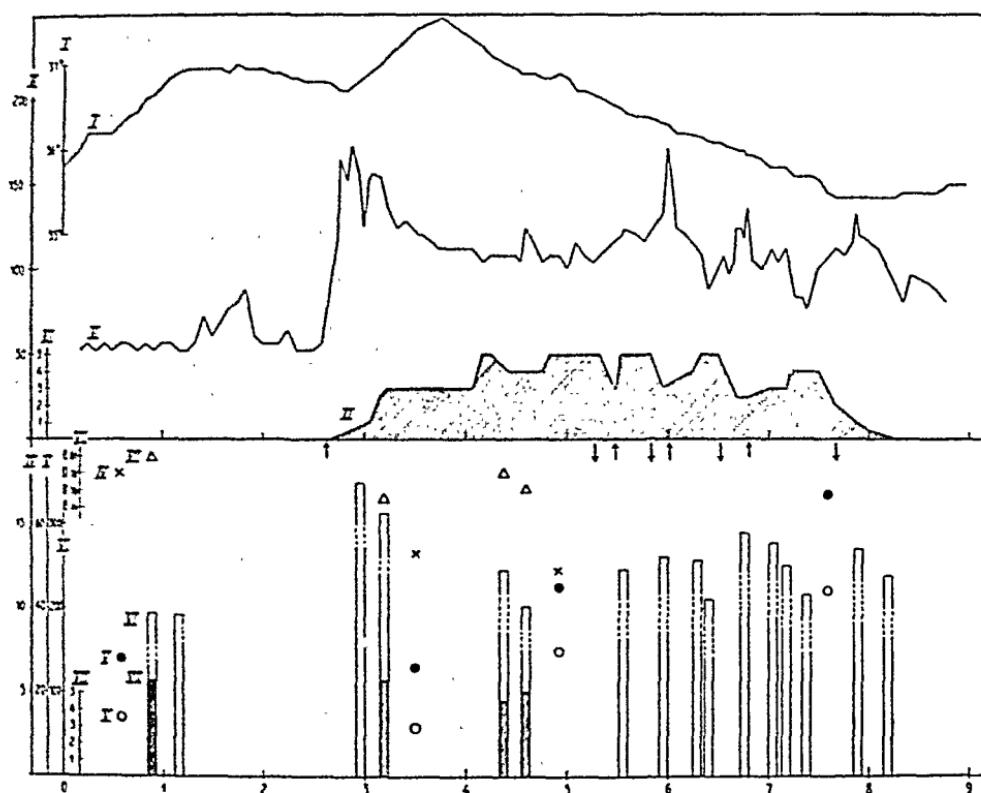


Abb. 17. Versuch vom 11.2.1941. Offenes Respirationssystem. Gewicht des Versuchstieres 680 gr. Urethan-Narkose  $2\frac{1}{2}$  Std. vor Beginn des Versuches eingeleitet. Das Tier atmete atmosphärische Luft ein. Das Asthma wurde durch 1%ige Histaminlösung hervorgerufen. Bei ↑ Beginn, bei ↓ Ende der Histamin-Inhalation.

Allgemeinzustand des Tieres: Ruhig.

- I. Rektaltemperatur in Grad Celsius.
- II. Atemfrequenz pro Minute.
- III. Grad des Asthmas.
- IV. Sauerstoff-Gehalt des arteriellen Blutes in Volumprozent.
- V. Milchsäure-Gehalt des arteriellen Blutes in mg% (bestimmt nach LEHMANN °, nach MILLER und MUNTZ •).
- VI. Lungenventilation in cc pro Minute (reduziert).
- VII. Respiratorischer Quotient.
- VIII. Sauerstoffaufnahme in cc pro Minute (reduziert).

Abszisse: Zeit in Stunden.

erreichte, stieg die Rektaltemperatur an. Später dagegen war sie während der ganzen Zeit, während der das Asthma schwer war, erniedrigt. Wenn das Asthma vorbei ist, zeigt die Rektaltemperatur eine Tendenz zum Anstieg.

*Die Sauerstoffsättigung des arteriellen Blutes.* Die Sauerstoffsättigung des arteriellen Blutes nimmt im Asthma ab. Abb. 17 zeigt, dass das arterielle Blut nicht mit Sauerstoff gesättigt ist, obgleich das Asthma nicht schwerer war, als dass die Temperatur steigen konnte. Bei schwererem Asthma ist das Blut nur zu ca. 50 % gesättigt. Dies stimmt mit den Werten überein, die MOORE und BINGER (1927) bei mechanischer Stenoseatmung fanden. Die beste Erklärung für die Ursache der Herabsetzung der Sauerstoffsättigung dürfte sein, dass der Gasaustausch in den Lungen erschwert ist.

*Der Milchsäure-Gehalt des Blutes.* Wir wissen, dass im anaphylaktischen Schock der Milchsäure-Gehalt des Blutes bei Meerschweinchen (ZUNZ und LA BARRE) wie beim Hund (McCULLOGH und O'NEILL) ansteigt. Letztere Verfasser bestimmten den Milchsäure-Gehalt in Äther-Morphin-Narkose und fanden eine Erhöhung um 100 %. Dabei zeigten sie, dass die Narkose selbst im Beginn des Versuches den Milchsäure-Gehalt im Blute um etwa 15 % steigerte. Nach zwei Stunden war der Milchsäure-Gehalt des Blutes wieder normal.

Geht man von den eben genannten Untersuchungen aus, so hat man alle Veranlassung, zu vermuten, dass auch im experimentellen Asthma der Milchsäure-Gehalt des Blutes erhöht sein wird. Dass dies in der Tat der Fall ist, konnte am experimentellen Histamin-Asthma in Urethannarkose nachgewiesen werden. Diese Versuche wurden nur in der Absicht durchgeführt, eine Vorstellung von der Grössenordnung des Milchsäure-Anstieges im Asthma zu gewinnen. In Übereinstimmung mit den Ergebnissen von McCULLOGH und O'NEILL dürfte auch die Urethan-Narkose keinen nennenswerten Einfluss auf den Milchsäure-Gehalt des Blutes ausüben. In dem in Abb. 17 wiedergegebenen Versuch ist dementsprechend der Milchsäure-Gehalt des Blutes am Zeitpunkt  $3\frac{1}{2}$  Std. normal (6 Stunden, nachdem die Narkose eingeleitet wurde). Zu dieser Zeit hatte das Versuchstier Asthma. Dies war aber so leicht, dass die Rektaltemperatur steigen

konnte. Als das Asthma schwerer wurde und die Rektaltemperatur sank, stieg der Milchsäure-Gehalt des Blutes an. Der Versuch in Abb. 17 zeigt, dass man bei leichterem Asthma keine Erhöhung des Milchsäure-Gehaltes zu finden braucht. In diesem Fall dürften demnach die Gewebe so viel Sauerstoff erhalten, wie sie benötigen, obgleich das Blut nicht voll mit Sauerstoff gesättigt ist. Wenn bei schwererem Asthma die Sauerstoffzufuhr zu den Geweben nicht mehr ausreichend ist, steigt der Milchsäure-Gehalt, wahrscheinlich infolge des Fehlens der Glykogen-Resynthese oder infolge der Verminderung der Milchsäure-Oxydation. In dem Versuch in Abb. 17 fanden wir eine Vermehrung der Milchsäure im Blut um etwa 30 mg %. Aus Tabelle 4 geht hervor, dass bei Meerschweinchen ohne Narkose der Milchsäure-Gehalt unter Sauerstoffmangel um 150 mg % ansteigen kann.

Die Milchsäure-Bestimmungen wurden nach LEHMANN, wie nach MILLER und MUNTZ ausgeführt. Letztere Methode ist eine Modifikation der in vielen Variationen bekannten Bestimmungsverfahren, die auf der Oxydation der Milchsäure zu Acetaldehyd beruhen. Diese Methode ist aber nicht spezifisch. Dies dürfte die Ursache dafür sein, dass die mit ihr gewonnenen Werte höher liegen, als die, die mit der von LEHMANN ausgearbeiteten enzymatischen Methode gefunden wurden. Es ist interessant, dass der Anstieg der Milchsäure-Werte im Asthma, der mit den verschiedenen Methoden gefunden wurde, gleich gross ist. Dies lässt uns annehmen, dass es sich hier ausschliesslich um eine Vermehrung der Milchsäure handelt, und nicht auch der unbekannten Substanzen, die mit der Methode von MILLER und MUNTZ neben der Milchsäure bestimmt werden.

*Der arterielle Blutdruck.* Das Symptomenbild, das beim Meerschweinchen durch Histamin ausgelöst wird, ist vor allem durch die kontrahierende Wirkung dieses Stoffes auf die Bronchialmuskulatur bestimmt. FELDBERG und SCHILF (1930, Seite 205) schreiben beispielsweise: »Bei anderen Tieren wirkt das Histamin im wesentlichen auf andere als die Kreislaufsorgane. So wirkt es beim Meerschweinchen vor allem auf die Bronchialmuskulatur, welche auf eine intravenöse Histamin-Injektion mit einem zur Erstickung führenden Bronchospasmus antwortet. Der Histamin-

schock des Meerschweinchens beruht somit auf einem Bronchialmuskelkrampf.»

Die Veränderungen, zu denen es im Kreislauf kommt, dürften nur sekundäre Folgeerscheinungen der Stenoseatmung sein.

Der arterielle Blutdruck steigt beim Meerschweinchen nach der Injektion von Histamin. Auf diesen Effekt dürfte allerdings die Art der Narkose einen gewissen Einfluss ausüben (vergl. FELDBERG und SCHILF, 1930, Seite 125 und 216). Im anaphylaktischen Schock wird der arterielle Blutdruck erhöht (u. a. SMITH, HARTER und ALEXANDER, sowie TOKUSIGE). TOKUSIGE betont, dass der arterielle Blutdruck erst dann sinkt, wenn bereits Atemstillstand eingetreten ist..

Nach den eben genannten Erfahrungen hat man alle Verlassung, anzunehmen, dass die Verhältnisse im experimentellen Asthma prinzipiell gleichartig sein werden. Es zeigte sich, dass dies in der Tat zutrifft. In einigen orientierenden Versuchen konnte ich während des experimentellen Asthmas eine Blutdrucksteigerung um 30 mm Hg oder weniger konstatieren. Diese hielt an, solange der Asthma-Anfall andauerte. Gleichzeitig mit dem Verschwinden des Asthmas kehrte auch der arterielle Blutdruck auf seinen Ausgangswert zurück. Diese Versuche wurden durchgeführt, um auszuschliessen, dass die Verminderung der Sauerstoffaufnahme, zu der es während des schweren Asthmas kommt, etwa auf einen Abfall des arteriellen Blutdruckes zu beziehen wäre. In einer späteren Arbeit beabsichtige ich, die Kreislaufverhältnisse während des experimentellen Asthmas eingehender zu studieren, dies vor allem deshalb, weil das experimentelle Asthma wesentlich bessere Möglichkeiten bietet, die Einwirkung der Stenoseatmung auf den Kreislauf zu untersuchen, als es der durch Injektion ausgelöste anaphylaktische Schock tut. Im experimentellen Asthma ist es nämlich möglich, leichter den Schweregrad und die Dauer der Stenoseatmung zu bestimmen. Auf diese, einer späteren Untersuchung vorbehaltenen Fragen, soll hier nicht näher eingegangen werden.

### Zusammenfassung.

Die wichtigsten Resultate, die in diesem Kapitel gewonnen wurden, sind: *Bei leichtem Asthma wird die Lungenventilation*

und die Sauerstoffaufnahme vergrössert, der respiratorische Quotient steigt etwas an. Bei schwererem Asthma können die Lungenventilation und die Sauerstoffaufnahme erheblich abnehmen und der respiratorische Quotient stark sinken, manchmal auf sehr niedrige Werte. Zwischen diesen beiden Extremen gibt es alle Übergänge. In der Nachperiode nach einem schweren Asthma, während dessen die Lungenventilation und die Sauerstoffaufnahme vermindert waren, findet man eine kompensatorische Erhöhung dieser beiden Grössen. Dies wird am deutlichsten in Versuchen an nicht betäubten Tieren.

Während des experimentellen Asthmas ist das Blut nicht mit Sauerstoff gesättigt und der Milchsäure-Gehalt des Blutes erhöht.

## KAP. VI.

# Die Gewebsatmung im experimentellen Asthma, sowie bei Herabsetzung des Sauerstoff-Gehaltes und bei Erhöhung des Kohlensäure-Gehaltes in der Atmungsluft.

Die Versuche über das Verhalten der oxydativen Prozesse in den Geweben wurden in der Regel so ausgeführt, dass abwechselnd Normalversuche und z. B. Asthma-Versuche jeweils an einer Serie von etwa 10 Versuchstieren angestellt wurden, die unter gleichen Bedingungen gehalten wurden. Alle Tiere mussten die letzten Stunden, bevor sie für die Anstellung der Versuche über die Gewebsatmung getötet wurden, im Respirationsapparat sitzen. Bei den meisten Kontrolltieren und allen Versuchstieren, bei denen ein Asthma erzeugt wurde, oder die einer Veränderung in der Zusammensetzung ihrer Atmungsluft ausgesetzt wurden, wurde die Sauerstoffaufnahme, sowie die Rektal- und Hauttemperatur bestimmt. (Die Untersuchung der Sauerstoffaufnahme unterblieb nur bei den Tieren, bei denen der CO<sub>2</sub>-Gehalt der Einatmungsluft erhöht wurde). Diese Versuchsanordnung diente dem Zwecke, möglichst zuverlässig den Zustand der Asthmatiere vor ihrem Tode mit der Grösse der Geweboxydationen in Beziehung setzen zu können. Das Respirationssystem hatte in diesen Versuchen niemals einen höheren Sauerstoff-Gehalt, als den der atmosphärischen Luft.

In den Asthma-Versuchen waren die Lungen blass und stark emphysematös. Die Schnittfläche war in der Regel trocken. Nur ausnahmsweise konnte an ihr eine gewisse Feuchtigkeit beobachtet werden, die auf einen leichten Grad von Ödem hinwies. Die Organe waren häufig deutlich cyanotisch, erschienen aber nicht geschwollen.

Die Gewebsatmung wurde vor allem in folgenden Geweben bestimmt: Leber, Oberschenkelmuskulatur (Vorderseite des Oberschenkels), Zwerchfell (nach Entfernung des sehnigen Teiles), Herzmuskel (nach Abschneiden der grossen Gefässe) und Niere (Rinde). An diesen Geweben wurde der Sauerstoffverbrauch im Warburg-Apparat sowie die Oxydations-Reduktions-Intensität im Methylenblau-Versuch untersucht.

In einer geringen Anzahl von Versuchen wurden ausserdem die Geweboxydationen im Gehirn (graue und weisse Substanz des Grosshirns), in der Brustmuskulatur, im Darm (unterer Teil des Dünndarms) und in der Lunge bestimmt. In diesen Versuchen wurde oft die Gewebsatmung nur mit einer der beiden oben genannten Methoden untersucht.

In der Regel wurden die Warburg- wie die Methylenblau-Versuche in Doppelbestimmungen durchgeführt. In den Methylenblau-Versuchen wurden häufig 3 bis 4 Röhrchen jeder Art gefüllt.

*Es ist wichtig, zu betonen, dass alle Unterschiede, die auf diese Weise in dem Verhalten der oxydativen Prozesse in den Geweben zu konstatieren waren, die von Tieren stammten, die ein Asthma hatten oder unter der Einwirkung einer Veränderung der Kohlensäure- oder Sauerstoffspannung in der Atmungsluft standen, ausschliesslich eine Folge der intra vitam entstandenen Veränderungen sein konnten. Nachdem die Versuchstiere getötet worden waren, wurden die Gewebe von den Kontroll- wie von den Astmatieren in gleicher Weise behandelt.*

### 1. Die Geweboxydationen im experimentellen Asthma.

Wie früher erwähnt, zeigten ABBERHALDEN und WERTHEIMER sowie BÜNGELER, dass die oxydativen Prozesse in den Geweben von Tieren, die im anaphylaktischen Schock starben oder getötet wurden, herabgesetzt sind. Diese Verfasser nehmen an, wie ebenfalls bereits weiter oben erwähnt, dass die Senkung der Gewebsatmung für den anaphylaktischen Schock spezifisch ist.

In der Absicht, festzustellen, inwieweit diese Ansicht zu Recht besteht, oder ob nicht auch bei anderen Formen der Stenoseatmung eine Herabsetzung der Gewebsatmung vorkommt,

untersuchte ich an Meerschweinchen die Gewebsatmung im experimentellen Asthma, das in der Regel durch Histamin ausgelöst wurde.

In den oben genannten Untersuchungen über die Geweboxydationen im anaphylaktischen Schock wurde nur der Sauerstoffverbrauch nach der Methode von Warburg bestimmt. Für die Beurteilung der Versuchsergebnisse ist es aber wertvoll, wenn man den Sauerstoffverbrauch mit der Oxydations-Intensität, gemessen mit der Methylenblau-Methode, vergleichen kann. Daher wurden Warburg- und Methylenblau-Versuche parallel durchgeführt.

Aus den angestellten Versuchen (siehe u. a. Abb. 23 und 24, sowie die Tabelle 7) geht hervor, dass man für die folgenden Gewebe eine Herabsetzung der Geweboxydationen sowohl mit der Warburg- wie mit der Methylenblau-Methode nachweisen kann: für die Leber, die Oberschenkelmuskulatur, das Zwerchfell und den Herzmuskel. In der Niere konnten mit den oben genannten Methoden keine Veränderungen der Geweboxydationen nachgewiesen werden. Im Gehirn waren keine sicheren Veränderungen nachweisbar. Am Darm wurde im Methylenblau-Versuch eine Herabsetzung der Geweboxydationen festgestellt, während im Warburg-Versuch keine sicheren Veränderungen gefunden wurden. Es muss aber betont werden, dass die erhaltenen Werte für den Sauerstoffverbrauch und für die Methylenblau-Entfärbungszeiten sehr ungleich waren; infolgedessen wurden die Versuche am Darm nicht weitergeführt.

Für die Brustmuskulatur kann eine deutliche Herabsetzung der Geweboxydationen im Methylenblau-Versuch nachgewiesen werden. Das Gleiche gilt für die Lunge, in der man vor allem im experimentellen Asthma, das auf anaphylaktischem Wege ausgelöst war, eine starke Senkung der Oxydo-Reduktions-Intensität sehen kann. Bestimmungen des Sauerstoffverbrauches im Warburg-apparat wurden an diesen Organen nicht ausgeführt, da mir nur eine begrenzte Anzahl von Warburg-Manometern zur Verfügung stand.

Die stärkste Herabsetzung der oxydativen Prozesse in den Geweben wurde bei langdauerndem und mässig schwerem Asthma gefunden, wobei die Körpertemperatur der Versuchs-

tiere allmählich immer mehr sank. Als Beispiel hierfür kann der in Abb. 19 wiedergegebene Versuch angeführt werden. Man sieht hier eine deutliche Herabsetzung der Gewebsatmung in der Leber, der Oberschenkelmuskulatur, dem Zwerchfell und der Herzmuskulatur. An der Niere dagegen wurden keine Veränderungen gefunden. Wie aus der Tabelle 7 hervorgeht, liess sich auch keine sichere Einwirkung auf das Gehirn feststellen. Zum Vergleich sind in Abb. 18 die Geweboxydationen von normalen Kontrolltieren am Tage vor und nach dem in Abb. 19 dargestellten Versuch wiedergegeben.

Bei kurzdauerndem Asthma erhält man in den meisten Organen weniger ausgesprochene Effekte, selbst wenn der Asthma-Anfall so schwer war, dass die Tiere starben. In Abb. 23 und 24 wurden daher die Stäbe, die ein Asthma von weniger als 5 Minuten Dauer representieren, von den anderen Versuchen getrennt. Diese Versuche wurden aber in die Berechnung der Mittelwerte und des mittleren Fehlers miteinbezogen.

In den Versuchen mit der stärksten Herabsetzung der Geweboxydationen zeigte sich, dass die Warburg- und Methylenblau-Versuche durchaus parallel liefen. In den Versuchen, in denen die Effekte weniger ausgesprochen waren, war häufig die Herabsetzung der Geweboxydationen in der einen von diesen beiden Methoden stärker ausgeprägt. In manchen dieser Versuche war demnach der Sauerstoffverbrauch nach der Warburg-Methode stärker herabgesetzt, in anderen dagegen die Oxydo-Reduktions-Intensität im Methylenblau-Versuch.

In einem Teil der Gewebe finden wir häufiger eine Herabsetzung der Geweboxydationen, als in anderen. Dies dürfte damit zusammenhängen, dass die verschiedenen Gewebe einen verschiedenen Grad von Empfindlichkeit gegenüber den schädlichen Einflüssen haben, denen sie im experimentellen Asthma ausgesetzt sind. Den stärksten Effekt finden wir an der Leber, während an der Niere überhaupt keine feststellbare Herabsetzung der Geweboxydationen zu beobachten ist. Weiter unten werden die Veränderungen der Gewebsatmung in einigen Organen eingehender beschrieben werden. Abb. 23 und 24 geben eine Zusammenstellung der Versuchsergebnisse von den Geweben, die am häufigsten untersucht wurden. Das Material in

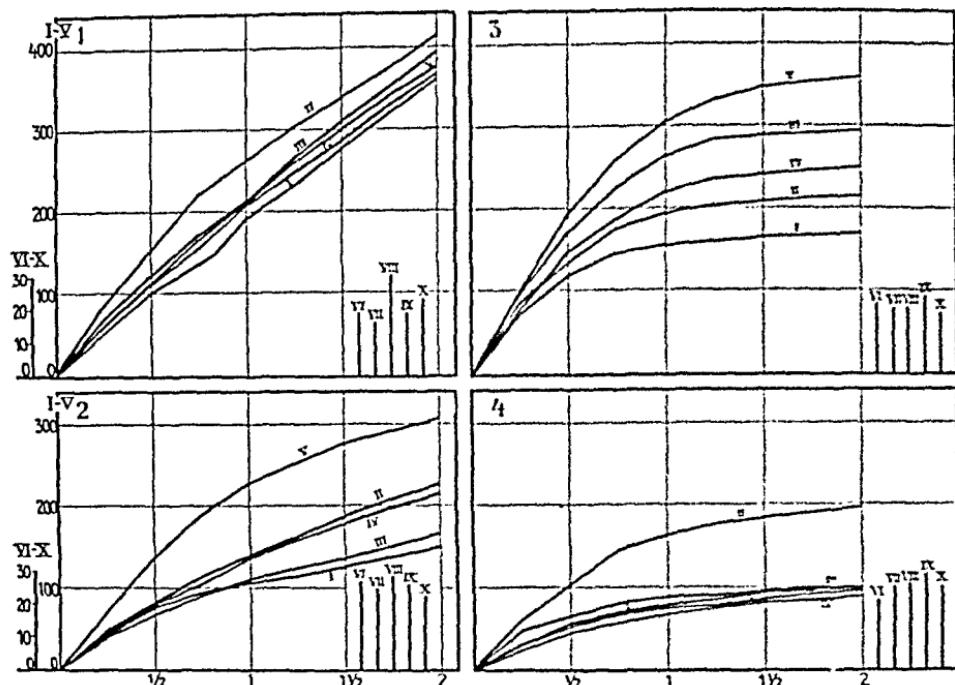


Abb. 18. Versuche vom 15. 11. bis 22. 11. 1941.

Gewebeoxydationen in den Organen von normalen Kontrolltieren.

Allgemeinzustand der Versuchstiere: Ruhig während des Versuches.

1. Niere. 2. Leber. 3. Oberschenkelmuskulatur. 4. Zwerchfell.

I bis V. Sauerstoffverbrauch im Warburg-Apparat in emm, gerechnet vom Beginn des Versuches.

VI bis X. Entfärbungszeiten von Methylenblau in Minuten.

Abszisse: Zeit in Stunden.

Versuch I und VI ausgeführt am 15. 11. 1941.

»	II	VII	»	»	17. 11. 1941.
»	III	VIII	»	»	18. 11. 1941.
»	IV	IX	»	»	19. 11. 1941.
»	V	X	»	»	22. 11. 1941.

(siehe auch Tabelle 7.)

Abb. 23 wurde statistisch behandelt, nicht aber das in Abb. 24, da es mit gewissen Schwierigkeiten verknüpft ist; die Entfärbungszeiten im Methylenblau-Versuch statistisch zu behandeln.

*Leber* (1 in Abb. 23 und 24): Von allen untersuchten Geweben findet man im Lebergewebe am regelmässigsten eine deutliche

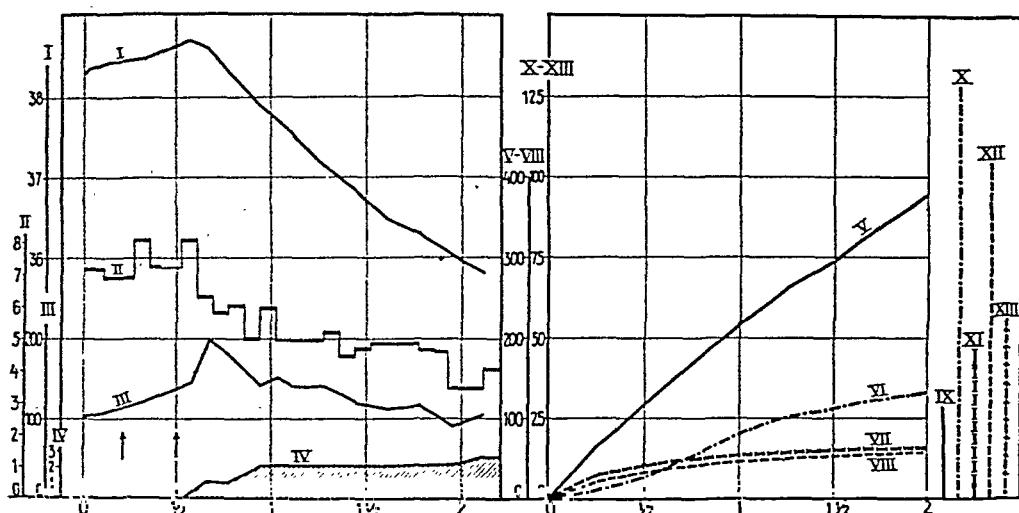


Abb. 19. Versuch vom 21. 11. 1941.

Respirationsversuch und Gewebsatmung im experimentellen Asthma.

Bei ↑ Nr. 1 Beginn der Einatmung einer 1 %igen Histamin-Lösung. Bei ↑ Nr. 2 Steigerung der Histamin-Konzentration auf 2 %.

Allgemeinzustand des Versuchstieres: Ruhig.

- I. Rektal-Temperatur in Grad Celsius.
- II. Sauerstoffverbrauch in cc pro Minute (reduziert auf 0°, 760 mm und Trockenheit).
- III. Atemfrequenz pro Minute.
- IV. Grad des Asthmas.
- V. Sauerstoffverbrauch im Warburg-Apparat in cmm für Niere.
- VI. > > > > > > Leber.
- VII. > > > > > > Oberschenkelmuskulatur.
- VIII. > > > > > > > Zwerchfell.
- IX. Entfärbungszeit von Methylenblau in Minuten für Niere.
- X. > > > > > > Leber.
- XI. > > > > > > Oberschenkelmuskulatur.
- XII. > > > > > > Zwerchfell.
- XIII. > > > > > > Brustmuskulatur.

Abszisse: Zeit in Stunden.

(siehe auch Tabelle 7.)

Herabsetzung der oxydativen Prozesse. In der Leber zeigt sich auch deutlich der Unterschied zwischen lang- und kurzdauern- dem Asthma, sowohl im Warburg- wie im Methylenblau-Versuch. Aus Abb. 23 geht hervor, dass die Unterschiede im Sauerstoff- verbrauch zwischen den Normal- und den Histamin-Versuchen, wie sie mit der Warburg-Methode gefunden wurden, statistisch sichergestellt sind.

*Oberschenkelmuskulatur* (2 in Abb. 23 und 24): Die Herab- setzung der Gewebeoxydationen ist hier nicht so gewöhnlich wie in der Leber. Die stärkste Senkung wird auch hier bei lang- dauerndem und mittelschwerem Asthma gefunden. Die Diffe- renz zwischen den Normal- und den Histamin-Versuchen ist auch hier statistisch gesichert. Im Methylenblau-Versuch sind die Unterschiede nicht so deutlich, wenn auch manche Versuche eine einwandfreie Verlängerung der Entfärbungszeit der Gewebe von asthmatischen Versuchstieren zeigen. Die Versuchsresultate lassen annehmen, dass in der Oberschenkelmuskulatur die gleichen Veränderungen auftreten, wie in der Leber, dass aber die Leber empfindlicher ist, als die Oberschenkelmuskulatur.

*Zwerchfell und Herzmuskel* (3 und 4 in Abb. 23 und 24): In den durchgeföhrten Versuchen konnte im Warburg- wie im Methylenblau-Versuch häufig eine Herabsetzung der oxydativen Prozesse konstatiert werden. Das Material, das unter der Ein- wirkung des Histamin-Asthmas untersucht wurde, ist aber nicht so gross, dass eine statistische Berechnung möglich wäre.

In den Normal-Versuchen ist das Herz stets dilatiert und schlägt meist noch bei der Entnahme aus dem Tier. Nach schwerem Asthma sieht man in der Regel, dass das Herz kontrahiert ist. Dies dürfte wahrscheinlich mit Veränderungen des intermediären Stoffwechsels zusammenhängen. In manchen dieser Fälle beobachtet man, dass die Versuchstiere ganz plötzlich sterben, ohne dass die Atmungsbehinderung merkbar zugenommen hätte. Es ist nicht ausgeschlossen, dass dieser plötzliche Tod durch Veränderungen im Atmungssystem des Herzmuskels verursacht sein könnte. Weitere Untersuchungen sollen diese Frage aufklären.

*Niere* (5 in Abb. 23 und 24): In der Niere findet man keine Herabsetzung der oxydativen Prozesse, selbst nicht in den Fäll-

len, bei denen die Geweboxydationen der übrigen untersuchten Organe am stärksten vermindert waren, was z. B. aus Abb. 19 hervorgeht:

Da das experimentelle Asthma in der Regel durch Histamin ausgelöst wurde, ist es angebracht, auf die Angaben der Literatur einzugehen, die über die Einwirkung von Histamin auf die Gewebsatmung vorliegen.

von EULER und LILJESTRAND (1929) untersuchten die Einwirkung von Histamin (in Konzentrationen von  $1:10^{-3}$  bis  $1:10^{-13}$ ) auf die Geweboxydationen der Froschmuskulatur im Methylenblau-Versuch. Sie fanden eine Erhöhung. Die optimale Konzentration war  $1:10^{-11}$ .

WOHLGEMUTH und SZORENYI (1933) untersuchten mit der Warburg-Methode die Histamin-Wirkung auf Gewebsschnitte vom Meerschweinchen (Leber, Niere, Haut und Gehirn) und beobachteten eine starke Erhöhung der Oxydationen. Die Steigerung war in der Leber und Haut in manchen Versuchen grösser als 100 %, in den übrigen Organen betrug sie etwa 25 %. Die den verschiedenen Rezipienten zugesetzte Histamin-Menge war 0,5 bis 2 mg.

Davon ausgehend, dass in der Anaphylaxie Histamin oder Histaminähnliche Substanzen freigesetzt würden, untersuchte BÜNGELER (1933) an Leberschnitten von Mäusen, denen Histamin injiziert wurde, die Gewebsatmung. Nach kleinen Dosen (2,5 mg subcutan) fand er eine geringe Steigerung der Geweboxydationen. Nach grossen Histamin-Dosen (80 bis 120 mg, Mäusen von 20 gr Gewicht subcutan oder intravenös injiziert) beobachtete er eine Herabsetzung der Gewebsatmung. BÜNGELER betont, dass er nach den grossen Histamindosen nur dann eine Senkung der Geweboxydationen fand, wenn die Versuchstiere schwere Symptome zeigten (Krämpfe, schwerste Dyspnoe, blaurote Verfärbung der Haut usw.). BÜNGELER untersuchte auch die Einwirkung von Histamin, das den Leberschnitten *in vitro* zugesetzt wurde. Dabei fand er bei einer Konzentration von  $1:2 \times 10^{-4}$  keinerlei Effekt, bei einer Konzentration von  $1:10^{-3}$  eine Hemmung um 10 %, bei einer Konzentration von  $1:2 \times 10^{-3}$  eine Hemmung um 25 %, und bei einer Konzentration von  $1:10^{-2}$  eine Hemmung um 35 %. Wie BÜNGELER annimmt, sprechen diese Versuche dafür, dass die Veränderungen, die er an Mäusen im anaphylaktischen Zustand nachweisen konnte, durch eine Histamin-Wirkung ausgelöst wurden. Es muss aber hervorgehoben werden, dass die Herabsetzung der Geweboxydationen, die nach der Injektion von Histamin in der Leber gefunden wurde, wahrscheinlich mit den schweren Symptomen zusammenhängt, die hierbei an den Versuchstieren auftraten. Hierauf wird in Teil 2 dieses Kapitels näher eingegangen werden. Was die Versuche mit Zusatz von Histamin zu den Gewebsschnitten betrifft, so ist zu bemerken, dass eine Hemmung erst bei sehr hohen Konzentrationen ( $1:2 \times 10^{-3}$ ) auftritt.

Ich untersuchte die Einwirkung des von mir verwendeten Histamins auf die Geweboxydationen der Leber im Warburg- und Methylenblau-Versuch. Dabei beobachtete ich keine sicheren Effekte. Die angewendeten Konzentrationen lagen zwischen  $1 : 10^{-3}$  und  $1 : 10^{-13}$ .

In meinen Versuchen wurden während einer Stunde in der Regel 2,5 mg Histamin vernebelt, häufig bedeutend weniger. Nur ein Teil dieser Menge kann vom Versuchstier aufgenommen werden sein. Ferner ist zu bemerken, dass die Veränderungen der Geweboxydationen stets von dem Schweregrad und der Dauer des Asthmas abhingen, und nicht von der Histamin-Konzentration, die nötig war, um das Asthma auszulösen. Unter Beachtung dieser Befunde und mit Rücksicht u. a. auf die quantitativen Gesichtspunkte, dürfte man daher berechtigt sein, anzunehmen, dass die im experimentellen Asthma gefundene Herabsetzung der Geweboxydationen nicht einfach eine Folge der Histamin-Einwirkung ist. Man hat vielmehr alle Veranlassung dazu, die Veränderungen der Gewebsatmung für eine Folge der Stenoseatmung selbst zu halten. Diese Verhältnisse werden ebenfalls in Teil 2 dieses Kapitels näher behandelt werden.

## 2. Die Geweboxydationen bei Herabsetzung des Sauerstoff-Gehaltes und Erhöhung des Kohlensäure-Gehaltes in der Atmungsluft.

In Kapitel IV und V wurde gezeigt, dass die Sauerstoffaufnahme bei schwererem Asthma bedeutend sinkt. In Kapitel V wurde ferner nachgewiesen, dass die Sauerstoffsättigung des arteriellen Blutes auch bei einem leichteren Asthma abnimmt. Dies konnte unter den fraglichen Bedingungen nur durch eine Erschwerung des Gasaustausches in den Lungen erklärt werden. Die Erschwerung des Gasaustausches in den Lungen dürfte ihrerseits zum Teil dadurch erklärlich sein, dass im schweren Asthma die Lungenventilation pro Minute abnimmt (Kapitel V). Infolgedessen sinkt die alveolare Sauerstoffspannung und die alveolare Kohlensäurespannung steigt an. Auch bei leichterem Asthma dürften wir mit einer Verschiebung der Zusammensetzung

ung der Alveolarluft in der eben erwähnten Richtung rechnen dürfen. Denn die Stenoseatmung verschlechtert an und für sich die Durchmischung der Atmungsluft (vergl. ROELSEN). Diese Annahme wird durch die Tatsache gestützt, dass der respiratorische Quotient sinken kann, obgleich sich die Lungenventilation im experimentellen Asthma erhöht (Abb. 17). Infolge der Erhöhung der alveolaren Kohlensäurespannung wird der Organismus mit Kohlensäure gesättigt, was sich in der Senkung des respiratorischen Quotienten im Beginn des Asthmas manifestiert (Kapitel V). *Zumindestens bei schwererem Asthma müssen wir daher damit rechnen, dass im Organismus die Sauerstoffspannung herabgesetzt, die Kohlensäurespannung erhöht ist.*

Wie in Abschnitt 1 dieses Kapitels ausgeführt wurde, kann die Herabsetzung der Geweboxydationen im experimentellen Histamin-Asthma nicht einfach als ein Histamin-Effekt erklärt werden. Man muss sich daher fragen: *Wäre es möglich, dass die durch die Stenoseatmung veränderten Gasspannungen im Organismus die primäre Ursache der Veränderungen in den Geweboxydationen darstellen?*

In der Literatur gibt es meines Wissens nur eine Angabe über Untersuchungen bezüglich der Geweboxydationen von Versuchstieren, die der Einwirkung von Gasgemischen ausgesetzt wurden, die von der atmosphärischen Luft abweichend waren. Im Zusammenhang mit ihren Untersuchungen über die oxydativen Prozesse in den Geweben von Meerschweinchen im anaphylaktischen Schock erwähnen ABBERHALDEN und WERTHEIMER (1922, Seite 498), dass die Gewebsatmung bei Tieren, die künstlich langsam erstickt worden waren oder infolge von Blutverlusten starben, normal war. Dies wird als Stütze für die Anschauung der Verfasser angeführt, dass die Herabsetzung der Geweboxydationen im anaphylaktischen Schock für diesen spezifisch wäre.

Um die oben gestellte Frage definitiv beantworten zu können, musste die Gewebsatmung untersucht werden, nachdem die betreffenden Versuchstiere unter der gleichzeitigen Einwirkung einer Herabsetzung der Sauerstoffspannung und einer Erhöhung der Kohlensäurespannung in der Atemluft standen. In einigen Versuchen wurden ausserdem die Geweboxydationen unter-

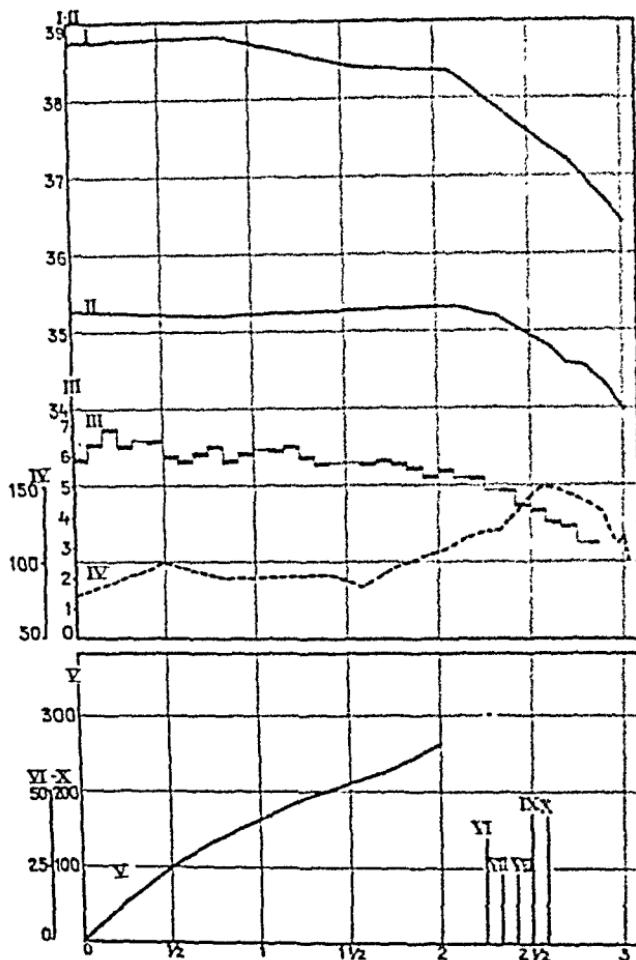


Abb. 20. Versuch vom 26. 2. 1943.

*Respirations-Versuch und Gewebsatmung bei Sauerstoffmangel.*

Allgemeinzustand des Versuchstieres: Ruhig. Während des Versuches tiefe, kräftige Atemzüge. Am Ende des Versuches sah das Tier matt aus. Zusammensetzung des Gasgemisches im System:

Zeit nach Beginn des Versuches	Kohlensäure- Gehalt	Sauerstoff- Gehalt
2 1/2 Std.	0,42 %	5,53 %
3     *	0,32 %	2,71 %

- I. Rektaltemperatur in Grad Celsius.
- II. Hauttemperatur in Grad Celsius.
- III. Sauerstoffaufnahme in cc pro Minute (reduziert).
- IV. Atemfrequenz pro Minute.

sueht, nachdem die Versuchstiere der Einwirkung allein einer Erhöhung der Kohlensäuretension oder der allein einer Senkung der Sauerstofftension ausgesetzt worden waren.

Die angewendete Methodik wurde in Kapitel III ausführlich beschrieben. Bei der Untersuchung der Einwirkung eines erniedrigten Sauerstoff-Gehaltes wurde der Sauerstoff-Gehalt im System allmählich gesenkt, so das der Sauerstoff-Gehalt am Schluss des Versuches etwa 3 bis 5 % betrug. Bei der Untersuchung der Einwirkung der Kohlensäure wurde der Kohlensäure-Gehalt im System auf 2 bis 12 % erhöht. Bei der Untersuchung der gleichzeitigen Einwirkung einer Senkung des Sauerstoff-Gehaltes und einer Erhöhung der Kohlensäure-Gehaltes enthielt das System am Ende der Versuche etwa 5 % Sauerstoff und 2 bis 5 % Kohlensäure.

Zunächst soll der Allgemeinzustand der Versuchstiere unter diesen verschiedenen Versuchsbedingungen kurz besprochen werden.

Bei Herabsetzung allein des Sauerstoffgehaltes im System steigt die Atemfrequenz allmählich von etwa 80 auf etwa 150 pro Min. Ist der Sauerstoffgehalt genügend gesunken, so nimmt die Atemfrequenz wieder ab (Abb. 20). Die Tiere wurden im allgemeinen aus dem Apparat genommen und getötet, wenn die Atemfrequenz auf etwa 100 pro Min. abgenommen hatte. Dies geschah, um die Versuchsbedingungen nicht unnötig zu komplizieren. Würde nämlich die Atemfrequenz sehr stark abnehmen, so müsste man damit rechnen, dass der Gasaustausch insuffizient würde, und die Kohlensäurespannung im Organismus ansteige. Wenn der Sauerstoffgehalt im System auf 5 bis 10 % abnahm, begann die Sauerstoffaufnahme sowie die Rektal- und Haut-

Abb. 20 Forts. *Versuch vom 26. 2. 1943.*

V. Sauerstoffverbrauch im Warburg-Apparat in cmm für Leber.

VI. Entfärbungszeiten von Methylenblau für Leber in Minuten.

VII.	>	>	>	>	>	+ m/300 Succinat.
VIII.	>	>	>	>	>	+ m/75 »
IX.	>	>	>	>	>	+ m/50 Lactat.
X.	>	>	>	>	>	+ m/15 »

Abszisse: Zeit in Stunden.

(siehe auch Tabelle 7.)

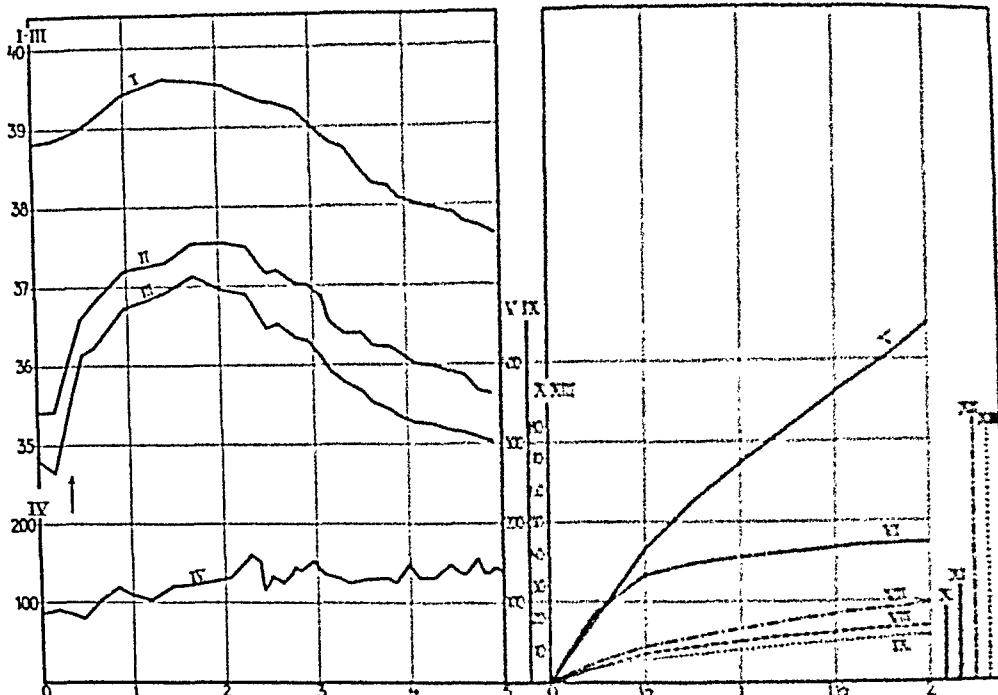


Abb. 21. Versuch vom 16. 2. 1942.

*Respirationsversuch und Geircbsatmung bei Sauerstoffmangel und Kohlensäure-Überschuss.*

Allgemeinzustand des Versuchstieres: Ruhig während des Versuches. Bei ↑ wurde die Zirkulationsgeschwindigkeit im System von 3,2 Liter pro Minute auf 0,16 Liter pro Minute geändert. Nach 1 Std. 25 Min. wurde der von dem Versuchstier verbrauchte Sauerstoff teilweise durch atmosphärische Luft ersetzt, wodurch der Sauerstoff-Gehalt im System allmählich abnahm. Zusammensetzung des Gasgemisches im System:

Zeit nach Beginn des Versuches	Sauerstoff- Gehalt	Kohlensäure- Gehalt
1 Std. 12 Min.	12,20 %	4,57 %
2 Std. 30 Min.	5,68 %	4,54 %
3 Std. 30 Min.	4,50 %	4,28 %

I. Rektaltemperatur in Grad Celsius.

II und III. Hauttemperatur in Grad Celsius an zwei verschiedenen Stellen.

IV. Atemfrequenz pro Minute.

V. Sauerstoffverbrauch im Warburg-Apparat in cmm für Niere.

VI. > > > > > > > Oberschenkelmuskulatur.

temperatur der Versuchstiere zu sinken, wie es der in Abb. 20 wiedergegebene Versuch zeigt. Gegen Ende des Versuches sieht das Tier matt und müde aus, es kann sich nicht mehr richtig aufrecht halten und schnappt nach Luft.

KOCH und HEYMANN (1938—1939) untersuchten in der Ruhe und während der Arbeit die Sauerstoffaufnahme von Versuchspersonen, die einer rasch entstehenden Verminderung des Sauerstoffgehaltes in der Einatmungsluft ausgesetzt wurden. Der Sauerstoff-Gehalt der Einatmungsluft wurde in 10 Minuten auf etwa 5 % herabgesetzt. Dies entspricht dem Partialdruck des Sauerstoffs in einer Höhe von ungefähr 10.000 m über dem Meerespiegel. Gegen Ende des Versuches wurden die Versuchspersonen zyanotisch, sie bekamen Muskelzuckungen, ließen den Kopf schlaff hängen und sackten zusammen. Bei aufrechtem Stehen begannen sie zu schwanken usw. Diese Symptome ähneln sehr den oben für die Meerschweinchen beschriebenen.

Bei einer Erhöhung des Kohlensäure-Gehaltes (um 3 bis 12 %) und bei gleichzeitig kaum verändertem Sauerstoff-Gehalt steigt die Atemfrequenz von ca. 80 auf ca. 150 pro Min. und bleibt dann während des ganzen Versuches unverändert. Die Atmung wird kräftiger und tiefer. Größere Veränderungen der Haut- und Rektaltemperatur sind nicht zu beobachten. Die Versuchstiere sehen weder matt noch müde aus. Sie wurden im allgemeinen nach 2 bis 3-stündiger Einwirkung der Kohlensäure getötet.

Bei gleichzeitiger Einwirkung einer Verminderung des Sauerstoff-Gehaltes und einer Erhöhung des Kohlensäure-Gehaltes verhalten sich die Versuchstiere annähernd ebenso wie bei reinem Sauerstoffmangel. Die Atemzüge werden kräftiger. Die

#### Abb. 21 Forts.

VII.	>	>	>	>	>	>	>	Zwerchfell.
IX.	>	>	>	>	>	>	>	Herzmuskel.
X.	Entfärbungszeit von Methylenblau (1,25 mg, 5 cc Lösung)	für Niere.						
XI.	>	>	>					für Oberschenkelmuskulatur.
XII.	>	>	>					Leber.
XIII.	>	>	>					Herzmuskel.

Abszisse: Zeit in Stunden.

(siehe auch Tabelle 7.)

Atemfrequenz steigt anfangs und beginnt gegen Ende des Versuches zu sinken (siehe Abb. 21). Wenn die Atemfrequenz auf 80 bis 100 pro Min. abgenommen hatte, wurden die Tiere in der Regel getötet. Die Zeit, die die Tiere unter der veränderten Gaszusammensetzung standen, betrug meist etwa 1  $\frac{1}{2}$  bis 3 Stunden. Gegen Ende der Versuche sank die Haut- und Rektaltemperatur der Tiere. Dies geht aus dem in Abb. 21 wiedergegebenen Versuch hervor. Die Versuchstiere sehen zu dieser Zeit matt und elend aus und schnappen mit jedem Atemzug nach Luft. Es kam manchmal vor, dass die Tiere bei einer Atemfrequenz von 140 pro Min. plötzlich starben. Seziert man die Tiere unmittelbar, nachdem sie zusammengebrochen waren, so findet man meist, dass das Herz stark kontrahiert ist, ebenso wie man es sieht, wenn die Tiere in einem Histamin-Asthma, das längere Zeit anhielt, starben.

Nach der Einwirkung von Sauerstoffmangel und nach der gleichzeitigen Einwirkung von Sauerstoffmangel und Kohlensäure-Überschuss waren die verschiedenen Organe bei der Sektion stark zyanotisch, sie zeigten aber im übrigen keine makroskopischen Veränderungen.

Die erste orientierende Versuchsserie, die über das Verhalten der Gewebsoxydationen unter den fraglichen Bedingungen durchgeführt wurde, ist in Abb. 22 wiedergegeben.

In dieser Serie wurden ein Normalversuch (14. 1.), zwei Versuche, in denen das Versuchstier allein dem Sauerstoffmangel ausgesetzt wurde (9. 1. und 16. 1.), ein Versuch, in dem das Tier der Einwirkung eines Kohlensäure-Überschusses unterworfen wurde (12. 1.), und schliesslich zwei Versuche, in denen die Tiere unter der gleichzeitigen Einwirkung des Sauerstoffmangels und des Kohlensäure-Überschusses standen (10. 1. und 13. 1.). angestellt. Bei der Analyse der in Abb. 22 wiedergegebenen Versuchsserie ergibt sich folgendes:

**Leber:** Abb. 22 Nr. 1 ergibt sich, dass die Gewebsatmung der Leber in den beiden Versuchen, in denen das Tier gleichzeitig der Einwirkung des Sauerstoffmangels und des Kohlensäure-Überschusses ausgesetzt war, deutlich vermindert war. Dagegen zeigten sich keine sicheren Veränderungen der Gewebsoxydationen in der Leber unter den übrigen Versuchsbedingungen. Es

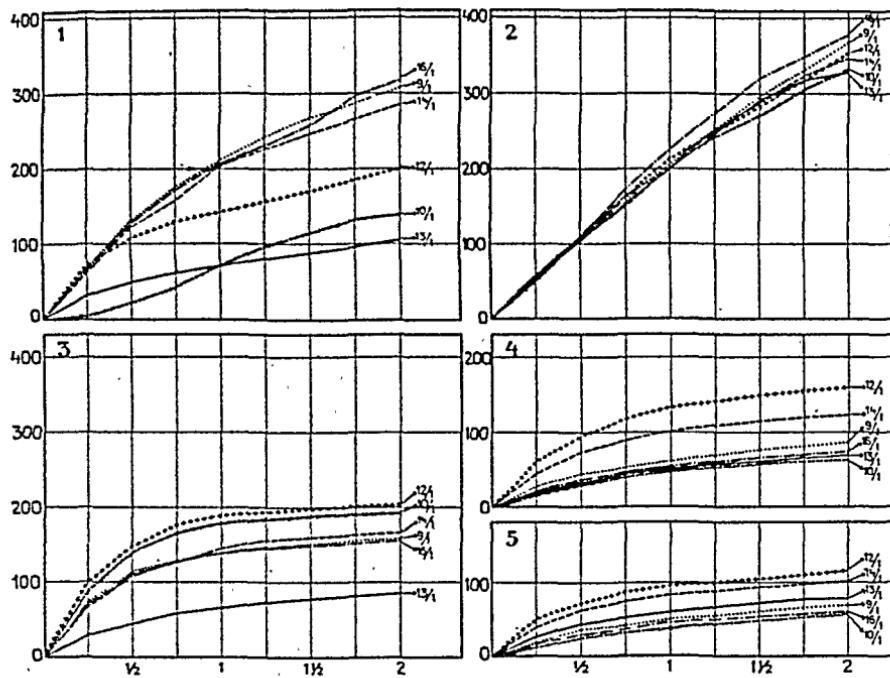


Abb. 22. Versuch vom 9. 1. bis 16. 1. 1942.

Gewebsoxydationen bei verschiedenem Kohlensäure- und Sauerstoff-Gehalt der Einatmungsluft.

Allgemeinzustand der Versuchstiere: Ruhig während des Versuches.  
 1. Leber. 2. Niere. 3. Oberschenkelmuskulatur. 4. Zwerchfell. 5. Herzmuskel.

(siehe auch Tabelle 7.)

fällt auf, dass der Kurvenverlauf in dem Versuch vom 10. 1. das gleiche Aussehen hat, wie es die Kurve der Sauerstoffaufnahme in dem in Abb. 19 wiedergegebenen Histamin-Versuch bietet.

**Oberschenkelmuskulatur:** Abb. 22 Nr. 3. Nur ein Versuch zeigt eine deutliche Herabsetzung der Gewebsoxydationen, nämlich der eine der Versuche, in denen das Versuchstier gleichzeitig der Einwirkung des Sauerstoffmangels und des Kohlensäure-Überschusses unterworfen wurde.

**Zwerchfell und Gehirn:** Abb. 22 Nr. 4 und 5. In diesen Organen sieht man keine so deutlichen Veränderungen der Gewebsoxydationen wie in der Leber. Vergleicht man aber die Befunde mit Abb. 18 Nr. 4, so erscheinen doch die Gewebsoxydationen bei Sauerstoffmangel wie bei Sauerstoffmangel und gleichzeitigem Kohlensäure-Überschuss ein wenig herabgesetzt.

*Niere:* Abb. 22 Nr. 2. Unter allen Versuchsbedingungen sind die Geweboxydationen normal und von gleicher Grösse, wie in den in Abb. 18 und 19 wiedergegebenen Versuchen. Ebenso wie die Geweboxydationen der Niere vom Histamin-Asthma nicht beeinflusst werden, scheinen auch unter den hier angewendeten Versuchsbedingungen die oxydativen Prozesse im Nierengewebe nicht verändert zu werden.

Die eben besprochene orientierende Versuchsserie schien darauf hinzuweisen, dass mindestens in bestimmten Organen die Geweboxydationen herabgesetzt werden, wenn die Versuchstiere vor ihrem Tode einer Veränderung in der Zusammensetzung der Einatmungsluft ausgesetzt wurden. Die Versuche der betreffenden Serie sprechen auch dafür, dass man in manchen Organen (Leber und Oberschenkelmuskulatur) bei der gleichzeitigen Einwirkung von Sauerstoffmangel und Kohlensäure-Überschuss leichter Veränderungen in den Geweben hervorrufen kann, die sich in einer Herabsetzung der oxydativen Prozesse manifestieren.

Da wir im experimentellen Asthma, wie weiter oben dargestellt, mit einer Herabsetzung der Sauerstoffspannung und Erhöhung der Kohlensäurespannung im Organismus rechnen müssen, untersuchte ich an einer grösseren Anzahl von Versuchstieren die Geweboxydationen, nachdem die Tiere der Einwirkung der beiden genannten Faktoren unterworfen wurden. Dies geschah, um ein genügend grosses Versuchsmaterial zu erhalten, das sichere Schlüsse zu ziehen erlaubte. Dagegen wurde nur eine geringere Zahl von Versuchen angestellt, in denen die Einwirkung des Sauerstoffmangels und des Kohlensäureüberschusses, jeder allein für sich, untersucht wurde. Die Ergebnisse der ausgeführten Versuche sind in Abb. 23 und 24 zusammengestellt. In diesen Versuchen wurden vor allem folgende Gewebe untersucht: Leber, Oberschenkelmuskulatur, Zwerchfell, Herzmuskel und Niere. *Wie aus Abb. 23 hervorgeht, ist bei gleichzeitiger Einwirkung von Sauerstoffmangel und Kohlensäure-Überschuss die Herabsetzung des Sauerstoffverbrauches im Warburg-Versuch für die Leber, die Oberschenkelmuskulatur, das Zwerchfell und den Herzmuskel statistisch sichergestellt. Dagegen sind die Geweboxydationen in der Niere nicht vermindert. Im allgemeinen*

gehen die Versuchsergebnisse bei der Bestimmung der Sauerstoffaufnahme nach Warburg vollkommen parallel mit den Befunden über das Verhalten der Oxydations-Intensität nach Thunberg.

In manchen Organen ist eine Senkung der Geweboxydationen häufiger zu beobachten, als in anderen. So werden die oxydativen Prozesse bedeutend häufiger in der Leber, im Zwerchfell und im Herzmuskel herabgesetzt, als in der Oberschenkelmuskulatur. Die Streuung der Werte für die Sauerstoffaufnahme der Oberschenkelmuskulatur ist erheblich grösser, als die Streuung der entsprechenden Werte für die übrigen Gewebe. Im Methylenblau-Versuch sind die Veränderungen hier nicht so deutlich, wie die in der Leber, dem Zwerchfell und dem Herzmuskel gefundenen.

Vergleicht man die Herabsetzung der Geweboxydationen, wie sie an den verschiedenen Geweben einerseits im experimentellen Asthma, andererseits unter der gleichzeitigen Einwirkung von Sauerstoffmangel und Kohlensäure-Überschuss beobachtet wurde, so findet man eine frappierende Übereinstimmung, die sich bis auf alle Einzelheiten erstreckt. Die Leber lässt die stärksten Wirkungen erkennen, die Niere überhaupt keine, die Oberschenkelmuskulatur zeigt die grösste Streuung der Werte usw.

*Dies dürfte zu dem Schluss berechtigen, dass mit grösster Wahrscheinlichkeit die Herabsetzung der Geweboxydationen im experimentellen Asthma auf der Senkung der Sauerstofften-  
sion und der Erhöhung der Kohlensäuretension beruht, die während des experimentelle Asthmas im Organismus zu beobachten sind.*

Die Herabsetzung der oxydativen Prozesse in den Geweben von Tieren, die im anaphylaktischen Schock getötet wurden, dürfte ebenfalls auf diese Weise zu erklären sein. Man kann selbstverständlich die Möglichkeit nicht vollkommen ausschliessen, dass die anaphylaktische Reaktion selbst eine Senkung der Geweboxydationen bewirken könnte. Es ist aber nicht angängig, die Herabsetzung der Geweboxydationen als spezifisch für den anaphylaktischen Schock anzusehen, wie es ABBERHALDEN und WERTHEIMER sowie BÜNGELER tun. Denn im Schock treten als Folge der Erschwerung des Gasaustausches in

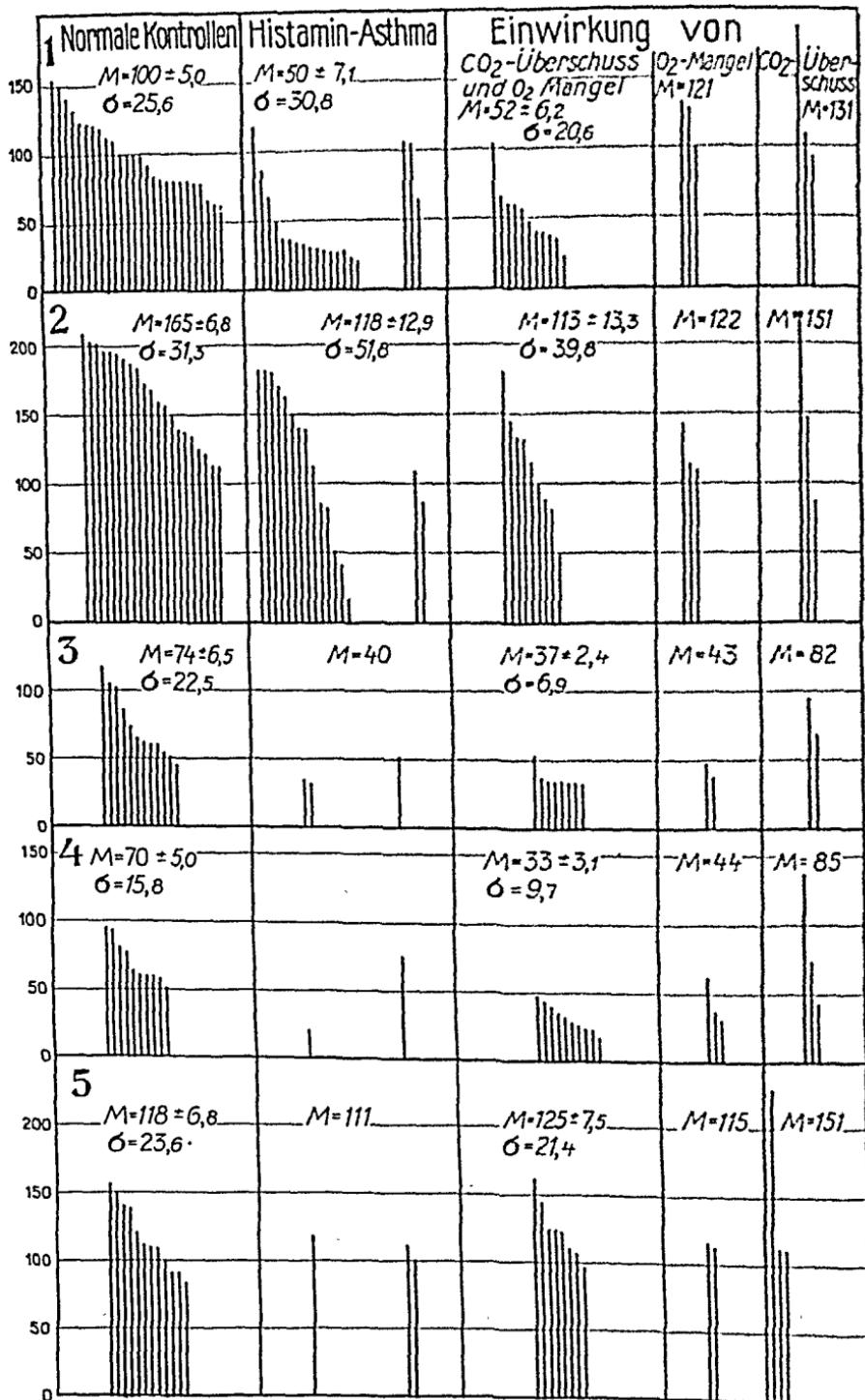


Abb. 23. (Siehe S. 132.)

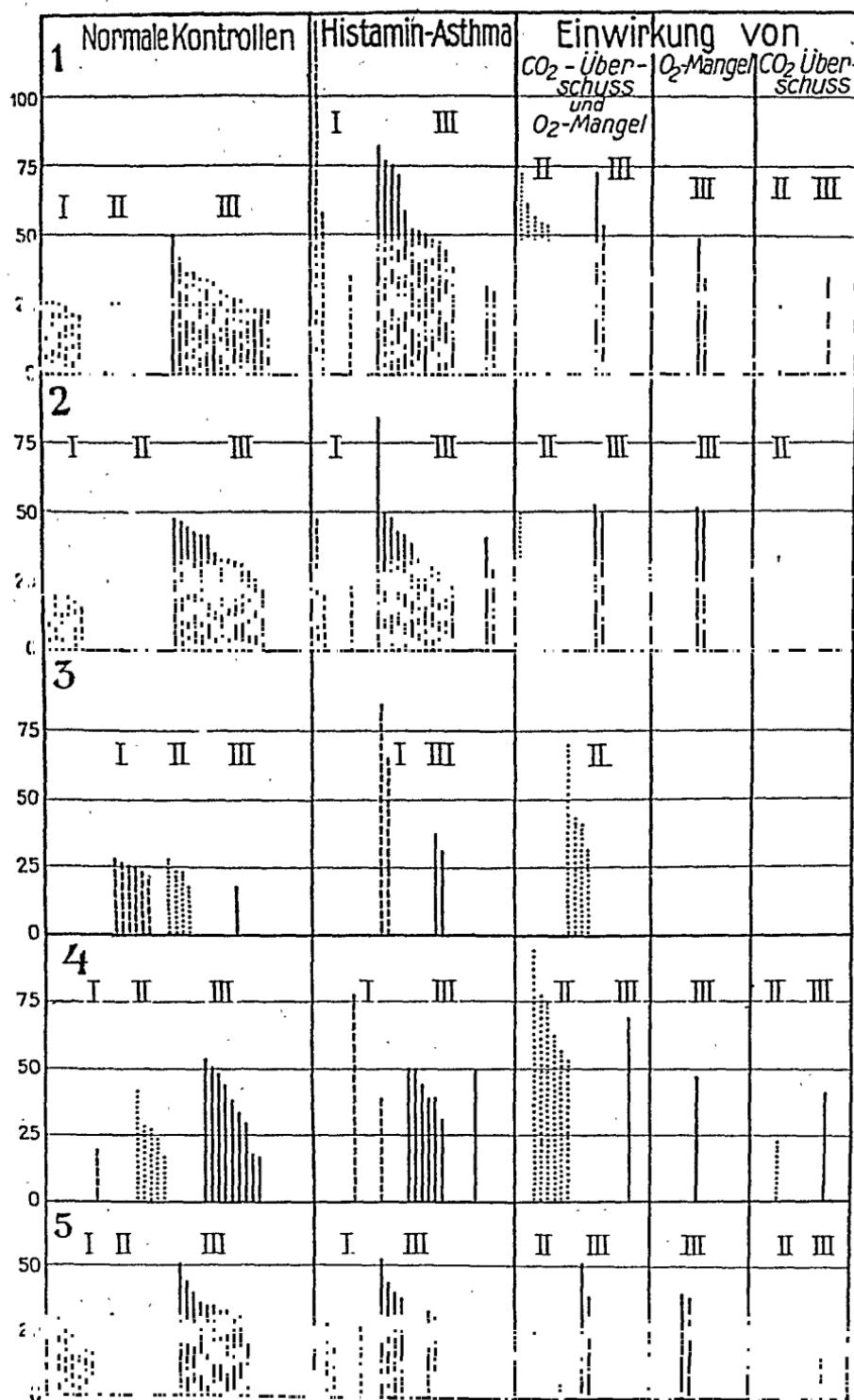


Abb. 24. (Siehe S. 132.)

Abb. 23. *Übersichtsbild der mit der Warburg-Methode ausgeführten Versuche.* Jeder Stab representiert einen Versuch. Die Stäbe sind nur nach ihrer Länge geordnet. In der Rubrik Histamin-Asthma wurden die Versuche, in denen der Asthma-Anfall weniger als 5 Minuten andauerte, in einer besonderen Gruppe zusammengestellt. Diese Versuche wurden aber in die Berechnung der Mittelwerte und der mittleren Fehler mitaufgenommen.<sup>1</sup>

1. Leber.
2. Oberschenkelmuskulatur.
3. Zwerchfell.
4. Herzmuskel.
5. Niere.

Ordinate: Sauerstoffaufnahme in cmm während der ersten halben Stunde.

Abb. 24. *Übersichtsbild über die ausgeführten Methylenblau-Versuche.* Jeder Stab representiert einen Versuch. Die Stäbe sind der Grösse nach geordnet.

In jeder Rubrik gibt es drei Gruppen:

Gruppe I: 1000 γ Methylenblau in 5 cc.

Gruppe II: 1250 γ       »       » 5 cc.

Gruppe III: 1000 γ       »       » 1,3 cc.

1. Leber.
2. Oberschenkelmuskulatur.
3. Zwerchfell.
4. Herzmuskel.
5. Niere.

Ordinate: Entfärbungszeiten in Minuten.

den Lungen Veränderungen der Sauerstoff- und Kohlensäuretension des Blutes auf, die ihrerseits gemäss den hier dargestellten Versuchen im Stande sind, die Geweboxydationen zu senken.

Wie früher erwähnt, konnte BÜNGELER (1934) nach grossen Histamin-Dosen nur dann eine Herabsetzung der Geweboxyda-

<sup>1</sup> Bei der Einwirkung von Kohlensäure enthält die Abb. einen durchgehend auffallend hohen Wert. Dieser stammt von einem Versuch, der nach Abschluss der übrigen Versuchsreihen mit einem albinotischen Tier, das von einem anderen Tierhändler geliefert wurde, ausgeführt wurde. Zu den früheren Versuchen wurden stets gefärbte Tiere verwendet. Es wäre möglicherweise richtiger, diesen Wert auszuschliessen, zumal da keine Kontrollversuche mit gleichartigen Tieren ausgeführt werden konnten.

tionen nachweisen, wenn gleichzeitig schwere Allgemeinsymptome (u. a. Dyspnoe) auftraten. Dies Verhalten kann in der oben besprochenen Weise leicht erklärt werden.

Abgesehen von den verschiedenen Formen der Stenoseatmung, dürfte man damit rechnen, dass auch bei allen anderen Zuständen, bei denen sich die Sauerstoffsättigung im Organismus in ausreichendem Grade vermindert und gleichzeitig die Kohlensäuretension erhöht, eine Veränderung in den Geweben entsteht, die sich in einer Herabsetzung der oxydativen Prozesse manifestiert. Dies gilt für alle Zustände, bei denen die Atmung oder der Kreislauf, sei es jedes dieser Systeme allein oder beide gleichzeitig, insuffizient sind.

Die von ABDERHALDEN und WERTHEIMER nachgewiesene Senkung der Geweboxydationen im anaphylaktischen Schock ist demnach nicht für diesen Zustand spezifisch, wie es von den genannten Autoren angenommen wurde. Die von jenen Verfassern gefundene Herabsetzung der Geweboxydationen hat aber durch die in dieser Arbeit vorgelegten Versuchsergebnisse einen erheblich weitgehenderen Inhalt erhalten.

Die Veränderungen der Gewebsatmung, die entstehen, wenn in der Einatmungsluft der Versuchstiere allein ein Sauerstoffmangel oder allein ein Kohlensäure-Überschuss hervorgerufen wird, haben für die Analyse der Herabsetzung der Gewebsatmung im experimentellen Asthma eine geringere Bedeutung. Denn wir finden, wie weiter oben auseinandergesetzt, beim experimentellen Asthma stets beide Faktoren gleichzeitig verändert. Ich untersuchte daher die Veränderungen der Geweboxydationen unter dem Einfluss allein des Sauerstoffmangels und allein des Kohlensäure-Überschusses nicht in so grossem Umfange, dass sichere Schlüsse gezogen werden könnten. Die Resultate der angestellten Versuche liegen aber in der Richtung, dass der Kohlensäure-Überschuss allein keine sicheren Veränderungen der oxydativen Prozesse in den Geweben bewirkt. Bei Sauerstoffmangel allein kann es anscheinend zu einer Verminderung der Gewebsatmung im Zwerchfell und im Herzmuskel kommen, während in der Leber keine sicheren Veränderungen festzustellen sind. *Bestimmte Gründe sprechen dafür, dass möglicherweise der Sauerstoffmangel für die Entstehung der Veränderungen in den Ge-*

*websoxydationen wesentlich ist, aber mindestens in manchen Fällen muss der Sauerstoffmangel durch einen gleichzeitigen Kohlensäure-Überschuss unterstützt werden.* Der Entstehungsmechanismus dieser Veränderungen soll in späteren Untersuchungen eingehend behandelt werden.

### 3. Die anaerobe Glykolyse.

In einigen Versuchsserien wurden Bestimmungen der anaeroben Glykolyse durchgeführt, vor allem in der Leber, da diese das Organ ist, in dem mit grösster Regelmässigkeit die stärkste Herabsetzung der Gewebeoxydationen feststellbar war. Diese Versuche wurden nur angestellt, um eine Vorstellung zu gewinnen, ob die Veränderungen der *in vitro* untersuchten anaeroben Glykolyse mehr oder weniger ausgesprochen waren, als die nach WARBURG und THUNBERG festgestellten Veränderungen der Gewebeoxydationen. Tabelle 3 gibt die erhaltenen Werte von einigen Versuchsserien wieder. Die angegebenen Werte stellen die Kohlensäure-Produktion von 200 mg Gewebshrei in der ersten Stunde dar (siehe auch Tabelle 7)

*Tabelle 3.*  
*Glykolyse-Versuche.*

Datum	Art des Versuches	cmm CO <sub>2</sub>	
26.1. 1943.	Normal	31	26
28.1. 1943.	Histaminasthma	47	48
29.1. 1943.	Histaminasthma	41	35
1.2. 1943.	Normal	33	33
2.2. 1943.	O <sub>2</sub> -Mangel + CO <sub>2</sub> -Überschuss	34	31
22.2. 1943.	O <sub>2</sub> -Mangel + CO <sub>2</sub> -Überschuss	24	19
23.2. 1943.	Normal	32	
26.2. 1943.	O <sub>2</sub> -Mangel	31	

Vergleicht man die gefundenen Werte für die Glykolyse mit den entsprechenden Veränderungen in den Gewebeoxydationen, so gewinnt man die Auffassung, dass die Veränderungen in der anaeroben Glykolyse, wenn man solche überhaupt feststellen

kann, weniger ausgesprochen und in ihrem Verlauf weit unregelmässiger sind, als die Veränderungen der Geweboxydationen. In dem schweren Histamin-Versuch vom 29. 1. liegen die Werte ein wenig über der Norm, in dem einen der Versuche unter der Einwirkung von Sauerstoffmangel und gleichzeitigem Kohlensäure-Überschuss, dem Versuch vom 22. 2., sind die gefundenen Werte etwas unter der Norm. In diesen beiden Versuchen waren die Geweboxydationen herabgesetzt, was sich am deutlichsten im Methylenblau-Versuch zeigte. Die eventuellen Veränderungen der anaeroben Glykolyse liegen demnach in einer solchen Grössenordnung, dass erst ein grosses Material ihre Feststellung gestatten würde.

Es ist nur schwer möglich, aus dem Verhalten der Glykolyse *in vitro* Schlüsse auf die Grösse der Glykolyse *in vivo* unter verschiedenen experimentellen Bedingungen zu ziehen. Man vermutete (MEYERHOF, 1937), dass nicht die gesamte in den Geweben analysierte Menge gewisser Co-Enzyme den enzymatischen Prozessen *in vivo* zugänglich wäre, bevor die Organstrukturen zerstört waren. Experimentelle Belege für diese Auffassung brachte ABDON (1942) bei, der für das überlebende Froschherz nachwies, dass nur ein kleiner Teil der als Adenosintriphosphorsäure analysierten Fraktion *in vivo* als Co-Phosphorylase dienen konnte.

Wie aus Kapitel V hervorgeht: ist der Milchsäure-Gehalt im Blut (und mit grösster Wahrscheinlichkeit auch im Gesamtorganismus) im anaphylaktischen Schock wie im experimentellen Asthma erhöht. Das Gleiche findet man bei Sauerstoffmangel und bei der gleichzeitigen Einwirkung von Sauerstoffmangel und Kohlensäure-Überschuss, wie aus Tabelle 4 hervorgeht.

Die Untersuchungen über den Milchsäure-Gehalt des Blutes lassen nur den Schluss zu, dass die Glykogen-Resynthese und die oxydativen Verbrennungen der Milchsäure nicht der Grösse der Milchsäure-Bildung entsprechen. Auf diese Weise entsteht *de facto* ein Überschuss an Kalorien, der einer Sauerstoffschuld entspricht. Inwieweit die Glykolyse absolut genommen erhöht oder gehemmt ist, kann nicht mit Bestimmtheit entschieden werden, da die Grösse der Resynthese und der oxydativen Verbrennungen der Milchsäure unbekannt ist. Nach Untersuchungen von O'NEILL, BING MOY und MANWARING (1925) nimmt beim Hund

Tabelle 4.

Datum	Art des Versuches	Milchsäure in mg%	Brenztraubensäure in mg%
8. 2. 43.	Normal	25	3,1
9. 2. 43.	Normal	25	2,5
10. 2. 43.	Leichtes anaphylaktisches Asthma	28	2,5
22. 2. 43.	Sauerstoffmangel und Kohlensäure-Überschuss		2,7
23. 2. 43.	Normal		3,2
26. 2. 43.	Sauerstoffmangel	154	5,4
3. 3. 43.	Sauerstoffmangel und Kohlensäure-Überschuss	174	

der Glykogen-Gehalt der Leber im anaphylaktischen Schock erheblich ab, wenn dieser Zustand länger als 10 Minuten andauert. Dies dürfte wahrscheinlich so gedeutet werden, dass eine Glykogenmobilisierung aus der Leber zu Gunsten anderer Organe stattfindet.

In Übereinstimmung mit den Ergebnissen arbeitsphysiologischer Untersuchungen sollte die Glykolyse im Beginn eines schwereren Asthmas vollkommen dem Ablauf der Glykolyse bei gewöhnlicher schwerer Muskelarbeit in ihrer ersten, partiell anaeroben Phase entsprechen, dem Zustand, den man in den ersten Minuten nach Beginn der Arbeit beobachten kann, bevor sich die Lungen und der Kreislauf den erhöhten Ansprüchen angepasst haben (siehe BANG, 1935). Im weiteren Verlauf eines experimentellen Asthmas oder unter der langdauernden Einwirkung eines Sauerstoffmangels und eines Kohlensäure-Überschusses wäre es nicht unmöglich, dass die Glykose *in vivo* gehemmt sein könnte. Am anoxybiotisch arbeitenden Froscherzen zeigte nämlich ABDON (1942), dass sich die Cophosphorylase-Aktivität *in vivo* rasch verschlechtert.

Es wäre möglich, durch Belastungsversuche mit Milchsäure, bzw. mit Glykose eine nähere Aufklärung über das Verhalten der Glykolyse *in vivo* beim experimentellen Asthma zu erhalten. Derartige Versuche mussten aber für eine spätere Arbeit zurückgestellt werden.

### Zusammenfassung.

In den meisten Geweben von Versuchstieren, die ein schwereres experimentelles Histamin-Asthma hatten, konnte eine Herabsetzung der Geweboxydationen nachgewiesen werden. In manchen Geweben aber, vor allem in der Niere, waren keinerlei Veränderungen zu konstatieren. Bei leichterem Asthma wurden keine sicheren Veränderungen der Geweboxydationen gefunden.

Die Geweboxydationen von Versuchstieren, die unter der Einwirkung einer Verminderung der Sauerstoffspannung und einer Erhöhung der Kohlensäurespannung in ihrer Einatmungsluft standen, waren herabgesetzt. Diese Senkung der oxydativen Prozesse stimmt bis in alle Einzelheiten mit der bei experimentellem Asthma gefundenen überein.

Da im experimentellen Asthma die Sauerstoffspannung im Versuchstier vermindert, die Kohlensäurespannung erhöht ist, kann mit grösster Wahrscheinlichkeit angenommen werden, dass diese veränderten Gasspannungen die Ursache für die Herabsetzung der Geweboxydationen darstellen, die man im Asthma findet.

Inwieweit in den Geweben von Tieren, die ein experimentelles Asthma hatten oder unter der Einwirkung einer Verminderung der Sauerstoffspannung und Erhöhung der Kohlensäurespannung in der Einatmungsluft standen, Veränderungen der anaeroben Glykolyse vorkommen, kann nach dem hier vorliegenden Material nicht mit Sicherheit entschieden werden. Die eventuellen Veränderungen würden aber weniger ausgesprochen sein, als die der Geweboxydationen.

## KAP. VII.

### Die Einwirkung einiger Substanzen auf die erniedrigte Gewebsatmung.

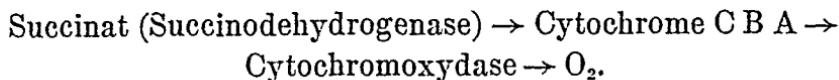
Im vorhergehenden Kapitel wurde gezeigt, dass bei Meerschweinchen, die unter der Einwirkung einer Verminderung der Sauerstoffspannung und einer Erhöhung der Kohlensäurespannung im Blute standen, die Gewebeoxydationen *in vitro* herabgesetzt sind. Man darf vermuten, dass diese Senkung der oxydativen Prozesse auf einer Störung der enzymatischen Prozesse in den Zellen beruht. Wäre dies in der Tat der Fall, so wäre es von grossem Interesse, festzustellen, ob die betreffende Schädigung generell ist, oder ob sie sich auf bestimmte enzymatische Systeme beschränkt.

In diesem Kapitel wird über Versuchsergebnisse berichtet werden, aus denen hervorgeht: In den Geweben von Versuchstieren, die unter der Einwirkung einer erhöhten  $\text{CO}_2$ -Spannung und einer herabgesetzten  $\text{O}_2$ -Spannung des Blutes standen, erwies sich ein bestimmtes Enzymsystem, *in vitro* untersucht, als ungeschädigt, während ein anderes geschädigt sein dürfte. Denn bei letzterem konnte eine Schädigung eines für dieses Enzymsystem notwendigen Coenzyms nachgewiesen werden.

Im ersten Abschnitt dieses Kapitels wird der Effekt behandelt, den der Zusatz von Laktat und von Succinat auf die Herabsetzung der Gewebeoxydationen ausübt. Im zweiten Abschnitt wird die Wirkung des Zusatzes von Cozymase und Cocarboxylase besprochen. Die Effekte wurden in der Regel nur an der Leber untersucht. Eine geringere Anzahl von Versuchen wurde auch an anderen Geweben durchgeführt, aber hier soll nur über die an der Leber ausgeführten Versuche berichtet werden.

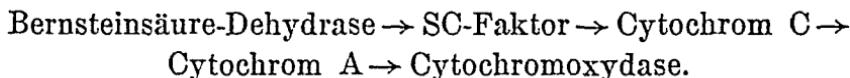
### 1. Die Einwirkung von Succinat und von Laktat.

Wie man früher im allgemeinen annahm, sollte sich die Oxydation der Bernsteinsäure durch tierisches Gewebe in folgender Weise abspielen:



Danach wäre kein Coenzym oder intermediärer Wasserstoffüberträger notwendig, um den Wasserstoff vom Succinat auf das Cytochrom C zu übertragen. Die Bernsteinsäure-Dehydrogenase würde demnach unter den tierischen Dehydrogenasen eine Sonderstellung einnehmen, indem sie unmittelbar mit dem Cytochrom-System reagieren könnte.

Aus neueren Untersuchungen von HOPKINS, MORGAN, LUTWAK-MANN (1939), STERN und MELNICK (1939), sowie STRAUB (1942) geht aber hervor, dass zwischen der Bernsteinsäure-Dehydrase und dem Cytochrom C ein aktivierendes Prinzip eingeschoben ist. STRAUB veranschaulicht die Umsetzungen in folgender Weise:



SC-Faktor bedeutet die Abkürzung für einen Succino-Dehydrase-Cytochrom C verbindenden Faktor. Die Natur dieses Faktors dürfte noch unbekannt sein.

Zum Unterschied von den Verhältnissen bei der Succinat-Oxydation ist u. a. für die Laktat-Oxydation die Anwesenheit von Coenzymen notwendig. Nach CORRAN, GREEN und STRAUB (1939) und STRAUB (1940) läuft die Laktat-Oxydation im Methylenblau-Versuch in folgender Weise ab:

1. Milchsäure + Cozymase  $\rightleftharpoons$  Brenztraubensäure + reduzierte Cozymase.
2. Reduzierte Cozymase + Diaphorase-Flavoprotein = Cozymase + reduziertes Diaphorase-Flavoprotein.
3. Reduziertes Diaphorase-Flavoprotein + Methylenblau = Diaphorase-Flavoprotein + Leukomethylenblau.

Man nahm an, dass die Cytochrome die physiologischen Äquivalente des Methylenblaus wären. Die Verhältnisse dürften aber in dieser Beziehung noch nicht völlig geklärt sein; denn reduziertes Diaphorase-Flavoprotein reagiert nach STRAUB nicht mit Cytochrom C. Auch wird das Diaphorase-Flavoprotein nicht durch C<sub>4</sub>-Dicarbonsäurekatalyse reoxydiert (vergl. STRAUB, 1942).

*Die Einwirkung des Zusatzes von Succinat und Laktat auf die Gewebeoxydationen.*

Nach Zusatz von Succinat in einer Endkonzentration von m/20 zum Lebergewebe im Normal-Versuch findet man eine starke Erhöhung der Sauerstoffaufnahme beim Warburg-Versuch (Abb. 26 und 27). Setzt man die gleiche Menge von Succinat dem Lebergewebe von Tieren zu, die unter der Einwirkung eines Kohlensäure-Überschusses und Sauerstoffmangels standen, und deren Sauerstoffaufnahme infolgedessen herabgesetzt war, so steigt die Sauerstoffaufnahme so stark an, dass sie ebenso gross wird, wie die des normalen Lebergewebes nach Succinatzusatz (Abb. 25 und 27). Die prozentuale Erhöhung der Sauerstoffaufnahme nach dem Zusatz von Succinat ist demnach erheblich stärker, wenn die Sauerstoffaufnahme des Lebergewebes vor dem Zusatz herabgesetzt war, als wenn sie vorher normal war.

Der Zusatz von Succinat zu normalem Lebergewebe im Methylenblau-Versuch erhöht die Oxydations-Intensität ein wenig. Die Erhöhung scheint in der Regel stärker zu sein, wenn das Succinat zu Lebergewebe zugesetzt wird, dessen Entfärbungszeit nach der Einwirkung eines Kohlensäure-Überschusses und Sauerstoffmangels auf die betreffenden Versuchstiere verlängert ist. In dem in Abb. 25 wiedergegebenen Versuch betrug z. B. die Entfärbungszeit für die Leber 83 Minuten, und für die Leber + Succinat 37 Minuten, während in dem in Abb. 26 wiedergegebenen Normal-Versuch die Entfärbungszeit der Leber 27 Minuten war, und die der Leber + Succinat 22 Minuten.

In Abb. 28 sind einige Versuche mit schwächeren Succinatkonzentrationen dargestellt. In ihnen ist der Effekt auf die Sauerstoffaufnahme erheblich geringer. Bei einer Endkonzentration von m/540 (II in Abb. 28) ist die Wirkung nur angedeutet,

bei einer Endkonzentration von m/108 dagegen erhält man einen deutlichen Effekt.

Bei einer Endkonzentration von m/108 wurde eine Succinatmenge zugesetzt, die einer Erhöhung der Sauerstoffaufnahme um etwa 320 cmm entsprechen würde, wenn alles Succinat in Fumarat umgesetzt würde. Die vollständige Verbrennung des gesamten bei dieser Konzentration zugesetzten Succinates würde einer Erhöhung der Sauerstoffaufnahme um etwa 2230 cmm entsprechen. Wie aus Abb. 28 hervorgeht, liegt die Steigerung der Sauerstoffaufnahme bei den mit dieser Succinat-Konzentration ausgeführten Versuchen zwischen etwa 100 und 230 cmm. Man muss allerdings damit rechnen, dass die in diesen Versuchen bestimmten Werte etwas zu niedrig waren, da die Erhöhung des Sauerstoffverbrauches in der Einstellungs-Periode nicht berücksichtigt wurde. Man dürfte aber doch feststellen können, dass die Erhöhung der Sauerstoffaufnahme nicht den Wert übersteigt, der einer vollständigen Verbrennung des zugesetzten Succinates entsprechen würde. Diese Versuche sprechen demnach nicht dafür, dass eine Bernsteinsäure-Katalyse anzunehmen wäre (siehe THUNBERG, 1943).

Setzt man Laktat in einer Endkonzentration von ca. m/25 dem Lebergewebe normaler Kontrolltiere im Warburg-Versuch zu, so findet man keinen oder nur einen unerheblichen Effekt auf die Sauerstoffaufnahme (Abb. 26 und 27).

Setzt man Laktat einem Lebergewebe zu, dessen Gewebsoxydationen infolge der Einwirkung von Sauerstoffmangel und Kohlensäure-Überschuss herabgesetzt sind, so findet man in manchen Fällen keinen, in anderen einen einigermassen deutlichen Effekt (Abb. 25, 27 und 28). Die Veränderung ist nicht so gross, dass die Sauerstoffaufnahme die Werte erreichen würde, die an Lebergewebe von normalen Kontrolltieren nach dem Zusatz der gleichen Menge Laktat zu beobachten sind.

In Normal-Versuchen mit der Methylenblau-Methode wird nach dem Zusatz von Laktat die Oxydations-Intensität etwas erhöht (Abb. 26, VII). In den Versuchen über die Gewebsoxydationen im Asthma bleibt dieser Effekt aber häufig aus (Abb. 25, VII). Dies dürfte darauf beruhen, dass unter diesen Bedingungen die Laktat-Konzentration bereits optimal ist.

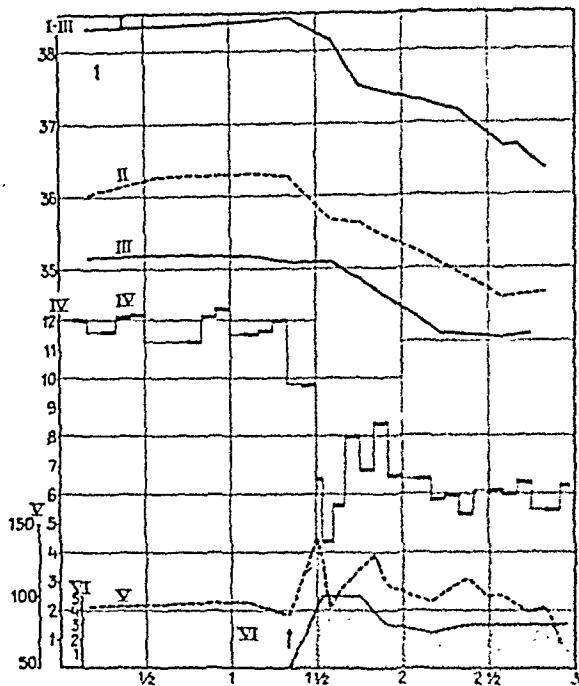


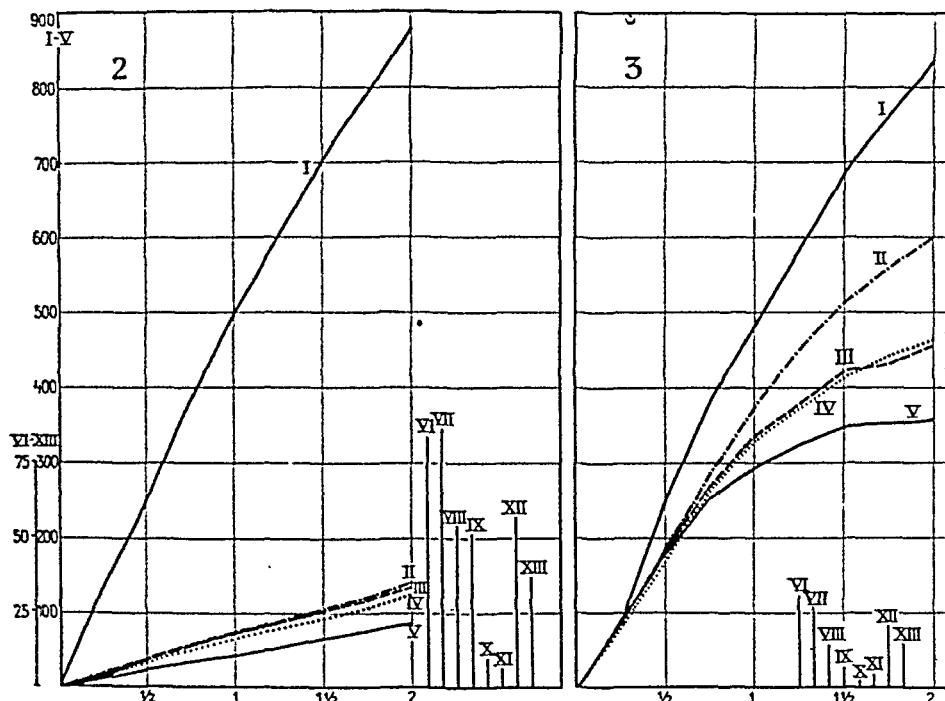
Abb. 25. Versuch vom 29. 6. 1942. Respirationsversuch und Gewebeoxydationen im Histamin-Asthma. Das Versuchstier atmete in atmosphärischer Luft. Asthma wurde durch 1 %ige Histamin-Lösung ausgelöst.  
Allgemeinzustand des Versuchstieres: Ruhig während des Versuches.

### 1. Histamin-Asthma im geschlossenen System.

- I. Rektaltemperatur in Grad Celsius.
- II und III. Hauttemperaturen in Grad Celsius.
- IV. Sauerstoffaufnahme in cc pro Minute (reduziert).
- V. Atemfrequenz pro Minute.
- VI. Schweregrad des Asthmas.

### 2. Gewebeoxydationen in der Leber.

- I. Sauerstoffaufnahme im Warburg-Apparat in cmm für Leber + m/22 Succinat.
- II. Sauerstoffaufnahme im Warburg-Apparat in cmm für Leber + m/27 Laktat + 1,5 mg Cozymase II.
- III. Sauerstoffaufnahme im Warburg-Apparat in cmm für Leber + m/27 Laktat + 0,5 mg Cozymase II.
- IV. Sauerstoffaufnahme im Warburg-Apparat in cmm für Leber + m/27 Laktat.



V. Sauerstoffaufnahme im Warburg-Apparat in cmm für Leber.  
VI. Entfärbungszeiten in Minuten für Leber.

VII.           »           »           »           »           » + m/10 Laktat.  
 VIII.          »           »           »           »           » + m/10 Laktat +  
               0,15 mg Cozymase II.  
 IX.           Entfärbungszeiten in Minuten für Leber + m/10 Laktat +  
               0,5 mg Cozymase II.  
 X.           Entfärbungszeiten in Minuten für Leber + m/10 Laktat +  
               1,5 mg Cozymase II.  
 XI.           Entfärbungszeiten in Minuten für Leber + m/10 Laktat +  
               3,0 mg Cozymase II.  
 XII.           Entfärbungszeiten in Minuten für Leber + m/10 Laktat +  
               3 mg Nicotinsäureamid.\*  
 XIII.          Entfärbungszeiten in Minuten für Leber + m/10 Laktat +  
               m/20 Succinat.

3. Geweboxydationen der Oberschenkelmuskulatur.  
Bezeichnungen wie bei der Leber.

(Siehe auch Tabelle 7.)

\* Die Zeit ist 76, nicht 57, wie in der Abb. angegeben.

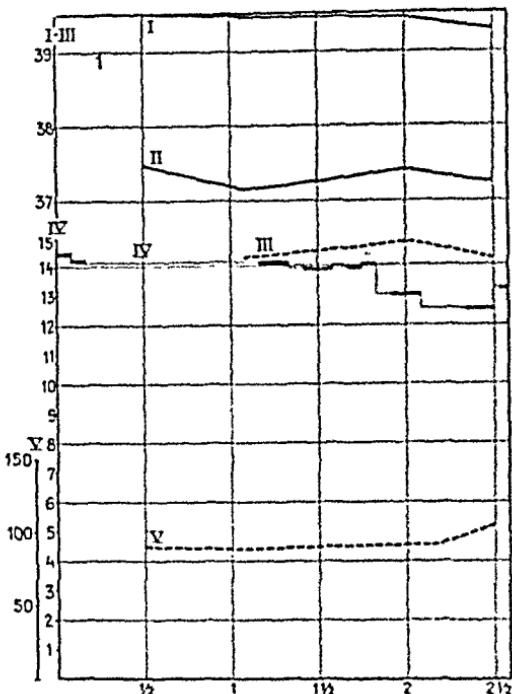


Abb. 26. Versuch vom 1. 7. 1942. Respirationsversuch und Geweboxydationen im Kontrollversuch zu Abb. 25. Bezeichnungen und Konzentrationen wie in Abb. 25, soweit nicht anders angegeben. Das Versuchstier atmete in atmosphärischer Luft.

Allgemeinzustand des Versuchstieres: Ruhig während des Versuches.

1. *Respirationsversuch.*

2. *Geweboxydationen der Leber.*

II. Sauerstoffaufnahme im Warburg-Apparat in cmm für Leber + m/27 Laktat + 3 mg Cozymase II.

III. Sauerstoffaufnahme im Warburg-Apparat in cmm für Leber + m/27 Laktat + 0,05 mg Cozymase II.

3. *Geweboxydationen der Oberschenkelmuskulatur.*

Bezeichnungen wie bei der Leber.

(Siehe auch Tabelle 7.)

Es ist interessant, dass man nach längerem Hunger (z. B. im Versuch vom 15. 12. 1942, in dem das Tier etwa 40 Std. gehungert hatte) eine Herabsetzung der Sauerstoffaufnahme der Gewebe im Warburg-Versuch finden kann. Der Ausfall des

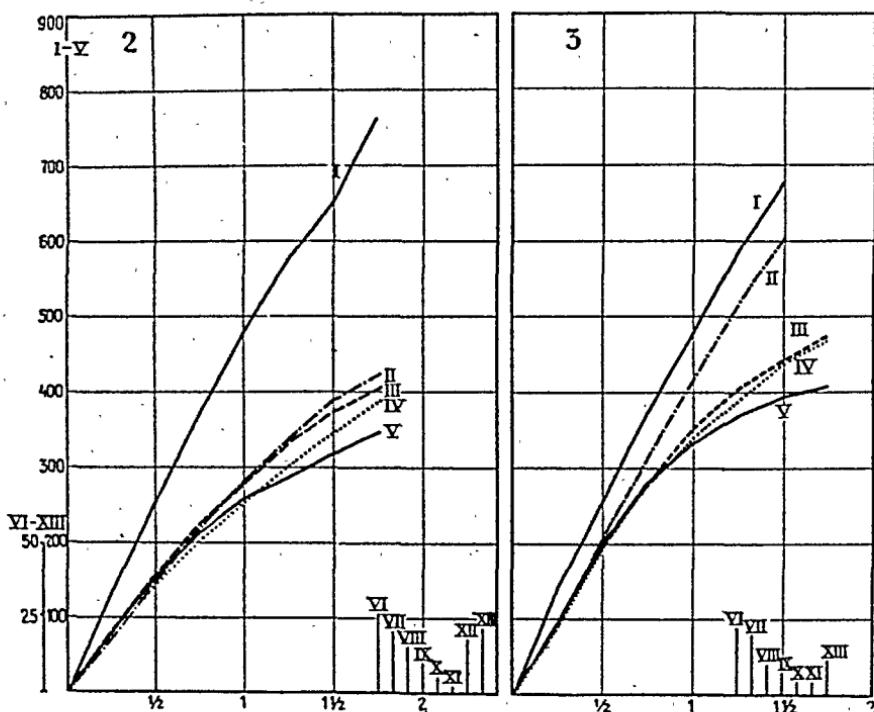


Abb. 26.

Methylenblau-Versuches scheint nicht in so hohem Grade durch den Hunger beeinflusst zu werden. In diesen Fällen beobachtet man vor allem im Warburg-, aber auch im Methylenblau-Versuch nach dem Zusatz von Laktat einen Effekt, der erheblich stärker ist, als der nach dem Zusatz von Laktat zu Geweben, deren Oxydations-Prozesse infolge der Einwirkung von Sauerstoffmangel und Kohlensäure-Überschuss herabgesetzt sind. Die Wirkung des Laktatzusatzes dürfte in diesen Fällen am besten dadurch zu erklären sein, dass hier ein Donator-Mangel besteht.

Aus den in diesem Abschnitt vorgelegten Versuchsresultaten können folgende Schlüsse gezogen werden:

Da die Succinat-Oxydation im Warburg-Versuch nicht geschädigt ist, kann man hieraus schliessen, dass das Cytochrom-System + Straub'schem SC-Faktor *in vitro* ebenfalls nicht geschädigt ist. Wie sich die Verhältnisse *in vivo* gestalten, lässt sich schwer beurteilen. Wie FORSSMAN (1941) zeigte, steigt die Succinat-Konzentration im Blut bei Sauerstoffmangel, dem das Versuchstier ausgesetzt wurde, erheblich an. Dies dürfte darauf

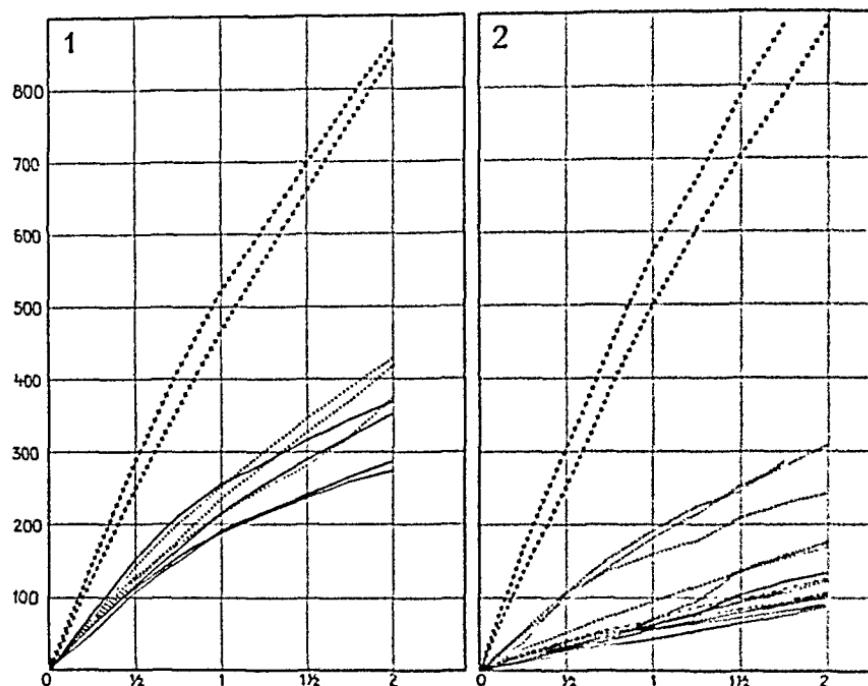


Abb. 27. Versuche über das Verhalten der Gewebeoxydationen bei Zusatz von grossen Mengen Succinat und Laktat. Endkonzentrationen waren für Laktat m/27 und für Succinat m/20.

1. Normalversuche.

- Kein Zusatz.
- ..... Zusatz von Laktat.
- + + + Zusatz von Succinat.

2. a. Asthma-Versuche.

- Kein Zusatz.
- ..... Zusatz von Laktat.
- + + + Zusatz von Succinat.

b. Sauerstoffmangel und Kohlensäure-Überschuss.

- — — Kein Zusatz.
- · — Zusatz von Laktat.

(Siehe auch Tabelle 7.)

hinweisen, dass unter den fraglichen Bedingungen die Succinat-Oxydation *in vivo* erschwert ist. Die einfachste Erklärung für diese Erscheinung dürfte die sein, die von FORSSMAN gegeben wurde, nämlich dass Sauerstoffmangel die Ursache für das Fehlen der Succinat-Oxydation *in vivo* wäre. In diesem Falle

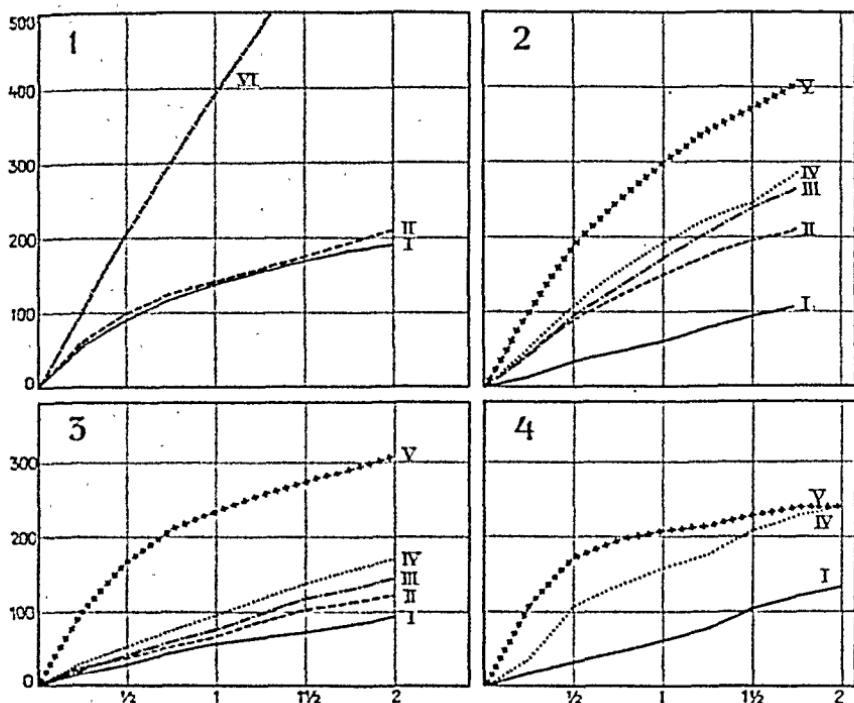


Abb. 28. Versuche über das Verhalten der Geweboxydationen bei Zusatz kleiner Mengen Succinat und Laktat.

1. Normalversuch vom 14. 12. 1942.
2. Histamin-Asthma vom 23. 12. 1942.
3. > > > 29. 12. 1942.
4. > > > 21. 12. 1942.

- I. Sauerstoffaufnahme im Warburg-Apparat in cmm für Leber ohne Zusatz.
- II. Sauerstoffaufnahme im Warburg-Apparat in cmm für Leber + m/540 Succinat.
- III. Sauerstoffaufnahme im Warburg-Apparat in cmm für Leber + m/135 Laktat.
- IV. Sauerstoffaufnahme im Warburg-Apparat in cmm für Leber + m/27 Laktat.
- V. Sauerstoffaufnahme im Warburg-Apparat in cmm für Leber + m/108 Succinat.
- VI. Sauerstoffaufnahme im Warburg-Apparat in cmm für Leber + m/54 Succinat.

(Siehe auch Tabelle 7.)

brauchte das Cytochrom-System in keiner Weise geschädigt zu sein. Diese Erklärung würde auch am besten zu den hier vorgelegten Versuchsergebnissen über das Verhalten der Gewebsoxydationen passen.

Die Schlüsse, die aus den Versuchen mit Laktat-Zusatz gezogen werden können, sind, dass die Herabsetzung der oxydativen Prozesse in den Geweben von Tieren, die unter der Einwirkung eines Sauerstoffmangels und eines Kohlensäure-Überschusses standen, nicht allein auf einem Laktatdefizit beruhen kann. Dies war auch *a priori* kaum anzunehmen, da im Sauerstoffmangel der Milchsäure-Gehalt des Organismus erhöht ist. Wie oben erwähnt, sind im Hungerzustand die Gewebsoxydationen herabgesetzt, sie werden aber nach Zusatz von Laktat wieder vollkommen normal. Vergleicht man die Gewebsoxydationen der Leber nach dem Zusatz von Laktat unter diesen beiden verschiedenen Versuchsbedingungen, so lässt sich folgendes sagen: In beiden Fällen ist Laktat im Überschuss vorhanden. Dass in dem zuerst genannten Falle die Gewebsoxydationen niedriger sind, als in dem zweitgenannten, beruht wahrscheinlich darauf, dass unter der Einwirkung des Sauerstoffmangels und Kohlensäure-Überschusses irgendein Faktor geschädigt wird, der für die Laktat-Oxydation notwendig ist. Im Abschnitt 2 dieses Kapitels wird dieses Problem eingehender behandelt.

## 2. Verhalten der Gewebsatmung bei Zusatz bestimmter Coenzyme, vor allem der Cozymase.

Im Methylenblau-Versuch an der Leber wird durch den Zusatz von Laktat die herabgesetzte Oxydations-Intensität, die man *in vitro* findet, wenn die Versuchstiere unter der Einwirkung eines Sauerstoffmangels und eines Kohlensäure-Überschusses standen, nur unwesentlich oder überhaupt nicht erhöht. Dies geht beispielsweise aus Abb. 25 hervor. Es ist daher von Interesse, zu untersuchen, inwieweit irgend ein anderer der Faktoren, die für den normalen Ablauf der Laktat-Oxydation notwendig sind, geschädigt sein könnte. In dem Schema, das STRAUB angab und das oben erwähnt wurde, werden außer

Laktat Cozymase und Diaphorase-Flavoprotein als notwendig bezeichnet.

In orientierenden Versuchen fand ich, dass durch Muskelkochsaft die herabgesetzte Oxydations-Intensität im Methylenblau-Versuch erheblich erhöht wurde. Da aber der Kochsaft u. a. sowohl Donatorsubstanzen wie Cozymase enthält, wendete ich bei der Weiterführung der Versuche nicht mehr Kochsaft an, sondern untersuchte den Einfluss der Donatorsubstanzen und der Cozymase, teils gemeinsam, teils jeden dieser Stoffe für sich, um unter besser definierten Bedingungen zu arbeiten.

Nach dem Zusatz von Cozymase zum Lebergewebe normaler Versuchstiere findet man eine deutliche Erhöhung der Oxydo-Reduktions-Intensität im Methylenblau-Versuch (siehe z. B. Abb. 26). Dagegen ist im Warburg-Versuch der Effekt kaum mehr als angedeutet. Nur gegen Ende des Versuches sieht man eine ganz geringe Erhöhung der Sauerstoffaufnahme, wie es auch Abb. 26 zeigt.

Setzt man die Cozymase einem Lebergewebe zu, dessen oxydative Prozesse infolge der Einwirkung von Sauerstoffmangel und Kohlensäureüberschuss auf die Versuchstiere herabgesetzt waren, so findet man im Methylenblau-Versuch einen sehr erheblichen Effekt. Dieser ist so stark, dass die Oxydations-Intensität bei der Anwendung genügend grosser Cozymase-Mengen die gleiche Höhe erreicht, die man am Lebergewebe normaler Versuchstiere beobachten kann, nachdem die gleichen Mengen Cozymase zugesetzt wurden. Dagegen war in der Sauerstoffaufnahme der Leber, untersucht nach WARBURG, bei den angewendeten Konzentrationen der Cozymase (bis zu 6 mg Cozymase II) kein sicherer Effekt feststellbar.

Die Wirkung des Cozymase-Zusatzes im Methylenblau-Versuch wird durch Abb. 29 verdeutlicht, in der 1 Normal-Versuch und 5 Versuche mit herabgesetzter Gewebsatmung dargestellt sind. Aus der Abb. geht zunächst hervor, dass die Oxydations-Intensität nach dem Zusatz grosser Mengen Cozymase in der Leber, deren oxydative Prozesse erheblich verlangsamt waren, ebenso gross ist, wie in der normalen Leber nach dem Zusatz der gleichen Menge Cozymase. Ferner zeigt sich, dass bei der Anwendung kleiner Mengen von Cozymase die Effekte in den Versuchen

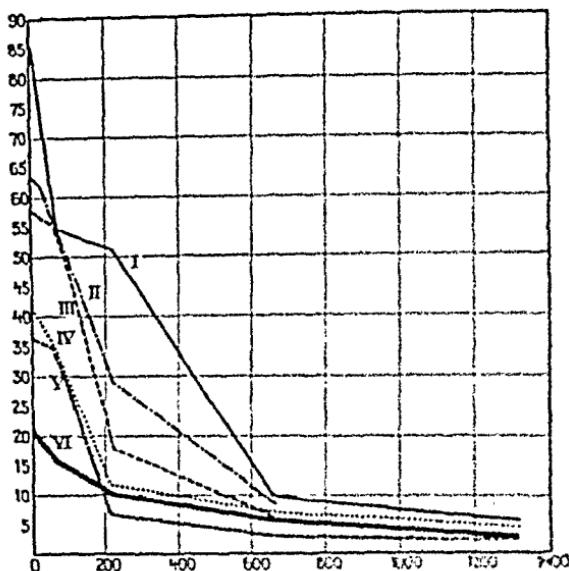


Abb. 29. Einwirkung von Cozymase im Methylenblau-Versuch.

Ordinate: Entfärbungszeiten in Minuten.

Abszisse: Zugesetzte Mengen Cozymase in  $\gamma$ . (Entsprechend Cozymase I.  
In den Versuchen wurde Cozymase II angewendet.)

Versuch 1. Ausgeführt am 29.6.1942. Histamin-Asthma.

>	2.	>	>	> 26.6.	>	>	>
>	3.	>	>	22.7.	>	>	>
>	4.	>	>	3.7.	>	>	>
>	5.	>	>	16.7.	>	Normal-Versuch.	
>	6.	>	>	1.7.	>	>	>

(Siehe auch Tabelle 7.)

stärker waren, in denen die Geweboxydationen herabgesetzt waren. Dies ganze Verhalten dürfte man am besten so erklären können, dass die Lebergewebe, die infolge der Einwirkung von Sauerstoffmangel und Kohlensäure-Überschuss eine Herabsetzung der oxydativen Prozesse zeigen, an einem Mangel an Cozymase leiden.

Nach den durchgeföhrten Versuchen hat man alle Veranlassung, anzunehmen, dass im Lebergewebe normaler Versuchstiere die Cozymase-Konzentration schon von Anfang an sicher nicht optimal ist, aber nicht so sehr weit unter den optimalen Werten liegt. Deshalb dürfte ein weiterer Zusatz von Cozymase keinen so grossen Effekt haben, wie es dann der Fall ist, wenn der Cozymase-Gehalt von Anfang an weit unter dem Optimum liegt.

In beiden Fällen darf man den stärksten Effekt erwarten, bevor die Cozymase-Konzentration in die Nähe des Optimums gelangt ist.

Wenn auch die ausgeführten Versuche sehr dafür sprechen, dass die hier vorgelegte Erklärung richtig ist, so kann diese Auffassung doch erst dann als voll bewiesen gelten, wenn es gelingt, zu zeigen, dass der Cozymase-Gehalt der Leber von Tieren, die unter der Einwirkung eines Sauerstoffmangels und eines Kohlensäure-Überschusses standen, niedriger ist, als der in der Leber von Normaltieren. Es zeigte sich, dass dies in der Tat der Fall ist, wie aus der folgenden Tabelle 5 hervorgeht, die alle im Januar und Anfang Februar 1943 ausgeführten Versuche mit Apozymase VI enthält.

Bei der statistischen Durchrechnung der in der Tabelle enthaltenen Serien wurde der Wert des Versuches vom 28. 1. 1943 nicht miteinbezogen, da das Versuchstier in diesem Versuch ein Asthma von weniger als 5 Minuten Dauer hatte. Wie früher nachgewiesen, ist unter diesen Umständen die Wirkung auf die Geweboxydationen, zumindestens was die Leber betrifft, erheblich schwächer.

Vergleicht man in der auf Seite 103 angegebenen Weise die Mittelzahlen des Cozymase-Gehaltes der Gewebe einerseits in den Normalversuchen und andererseits in den Versuchen, in denen die Tiere ein Histamin-Asthma hatten oder unter der Einwirkung von Sauerstoffmangel und Kohlensäure-Überschuss standen, so findet man, dass weniger als 1 % Wahrscheinlichkeit dafür besteht, dass die gefundenen Unterschiede auf einem Zufall beruhen könnten.

Was das Verhältnis zwischen oxydierter Cozymase und Dihydrocozymase betrifft, so zeigen sich grosse Differenzen zwischen den verschiedenen Versuchen. Dies steht in guter Übereinstimmung mit früheren Untersuchungen (v. EULER und Mitarbeiter, 1938). Diese Verfasser nehmen an, dass die Variationen auf enzymatischen Veränderungen bei der Extraktion beruhen. Es wäre aber möglich, dass die Veränderungen unter bestimmten Bedingungen ein Ausdruck für den »Redox-Zustand« wären, wie er zur Zeit vor dem Eintritt des Todes im Organismus herrschte. Hierfür spricht, dass ich in der Regel bei den Tieren, die in einem schweren Asthma sehr rasch starben, und bei

Tabelle 5.

Datum	Art und Dauer der Versuche	γ Cozymase pro gr frisches Gewebe nach Extraktion mit		Oxydierte Cozymase; Dihydro-Cozymase		Oxydierte Cozymase; Dihydro-Cozymase
		Phosphat Puffer	Destilliertem H <sub>2</sub> O	n/20 NaOH (Oxydierte Cozymase)	n/20 HCl (Dihydro-Cozymase)	
5. 1. 1943	Normal	468	312	139	120	259
7. 1. 1943	+	552	156	154	12	337
15. 1. 1943	+	431	162	216	0.5	312
19. 1. 1943	+	384	162	120	—	—
20. 1. 1943	+	422	303	161	12	344
26. 1. 1943	+	573	310	—	—	—
1. 2. 1943	+	—	301	321	0.2	462.5
		M = 172 ± 75	M = 454 ± 64	M = 172 ± 75	M = 454 ± 64	M = 319
10. 1. 1943	Histamin-Astma	40°	252	10.5	14.6	254
13. 1. 1943	+	66°	243	16.0	11.5	21.5
28. 1. 1943	+	—	159.4	—	26.1	20.7
29. 1. 1943	Co <sub>3</sub> Mang. Co <sub>3</sub> -Chloroet. Co <sub>3</sub> -Chloroet. Co <sub>3</sub> -Chloroet.	—	—	—	1.5	17.1
30. 1. 1943	Co <sub>3</sub> Mang. Co <sub>3</sub> -Chloroet. Co <sub>3</sub> -Chloroet. Co <sub>3</sub> -Chloroet.	—	—	—	—	27.0

denen ein excessiver Sauerstoffmangel im Organismus herrschen musste, sehr niedrige Werte für das Verhältnis Cozymase: Dihydrocozymase fand. Dies Verhalten wird durch den Versuch vom 28. 1. 1943 in der Tabelle 5 belegt.

Die Summe aus oxydierter Cozymase und Dihydrocozymase ist in den Normalversuchen erheblich konstanter, und in allen Versuchen etwas niedriger, als die Werte, die man durch die Extraktion mit dest. Wasser oder Phosphat-Puffer erhält. Dies steht ebenfalls in Übereinstimmung mit den Befunden von v. EULER und seinen Mitarbeitern, die der bei der Neutralisierung zu Stande kommenden Salzbildung die fragliche hemmende Wirkung zuschreiben. SCHLENK (1941) beziffert diese Hemmung der Vergärung auf bis zu etwa 20 %. In den Versuchen mit schwerem Asthma und langdauernder Einwirkung von Sauerstoffmangel und Kohlensäure-Überschuss ist es aber auffallend, dass die Summe aus oxydierter Cozymase und Dihydrocozymase erheblich (bis zu 100 %) niedriger ist, als die für die totale Cozymase gefundenen Werte. Dies zeigt der Versuch vom 29. 1. 1943. Es ist im Augenblick nur schwer möglich, sich über die Ursache dieses Verhaltens zu äussern.

In meinen Versuchen wendete ich, wie früher erwähnt, drei verschiedene Cozymase-Präparate an, von denen das eine (Cozymase I) als reine Cozymase angesehen werden kann. Man fragt sich, ob die Stoffe, die in den beiden anderen Cozymase-Präparaten als Verunreinigungen enthalten sind, irgendeine Wirkung auf die Geweboxydationen ausüben könnten. Um diese Frage aufzuklären, stellte ich einige Versuche an, in denen die verschiedenen Cozymase-Präparate im gleichen Versuch miteinander verglichen wurden. In Abb. 30 wurden in einem Versuch mit CO<sub>2</sub>-Überschuss und O<sub>2</sub>-Mangel und in einem Normalversuch dem Lebergewebe verschiedene Mengen Cozymase I und Cozymase III zugesetzt. 125 γ Cozymase I enthalten, wie früher in Kapitel III nach dem Ausfall des Vergärungsversuches angegeben, ebensoviel Cozymase, wie 735 γ Cozymase III. In diesen Versuchen wurde keine Donatorsubstanz zugesetzt. Wie aus Abb. 30 hervorgeht, dürfte die stimulierende Wirkung auf die Intensität der Oxydationen fast aus-

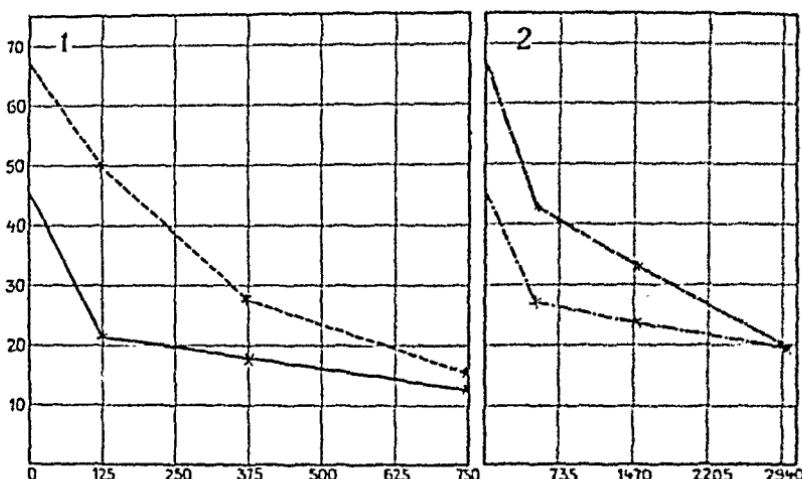


Abb. 30. Methylenblau-Versuche mit Cozymase I und III.

1. Zusatz von Cozymase I.
2. »   »   »   III.

Die beiden oberen Kurven in 1 und 2 gehören zum Versuch vom 22.2. 1943 ( $\text{CO}_2$ -Überschuss und  $\text{O}_2$ -Mangel).

Die beiden unteren Kurven in 1 und 2 gehören zum Versuch vom 23.2. 1943 (Normalversuch).

Ordinate: Entfärbungszeiten in Minuten.

Abszisse: bei 1.  $\gamma$  Cozymase I, bei 2.  $\gamma$  Cozymase III.

schliesslich dem Cozymase-Gehalt der Präparate zuzuschreiben zu sein.

In einigen Versuchen untersuchte ich auch die Einwirkung von Cozymase bei verschiedenem Gehalt an Methylenblau. Diese Versuche wurden durchgeführt, um auszuschliessen, dass nicht etwa eine längere Entfärbungszeit im Methylenblau-Versuch an und für sich mit einem kräftigeren Cozymase-Effekt einhergehe. Es zeigte sich aber, dass dies nicht der Fall ist, wie aus dem folgenden Versuch hervorgeht:

#### *Versuch vom 25.3.1943.*

In der einen der Serien betrug der Methylenblau-Zusatz 1000  $\gamma$ , in der anderen 1500  $\gamma$ . Bei beiden Serien wurde Cozymase III in verschiedenen Konzentrationen zugesetzt. Kein Zusatz von Donator-Substanz. Siehe im übrigen Tabelle 6.

Tabelle 6.

Zugesetzte Menge Cozymase III in γ:	Entfärbungszeiten von Methylenblau in Min. für	
	1000 γ:	1500 γ:
—	37 u. 40	47 u. 50
500	37,5	—
1000	31,5	34
3000	21,5	34,5
4000	19	36

Ich untersuchte im Methylenblau-Versuch einige Substanzen, von denen man sich denken könnte, dass sie beim Zerfall der Cozymase entstehen und eine Cozymase-ähnliche Wirkung haben könnten. Es galt hierbei, der Frage nachzugehen, ob die gefundenen Veränderungen durch die Cozymase selbst oder etwa durch ihre möglichen Zerfallsprodukte ausgelöst würden. Die untersuchten Substanzen waren Adenylsäure, Adenosin, Adenin und Nicotinsäure-Amid. In Abb. 31 sind die Ergebnisse von zwei Versuchen wiedergegeben. Der eine ist ein Normalversuch (1), der andere ein Versuch, in dem das Versuchstier unter der gleichzeitigen Einwirkung von Sauerstoffmangel und Kohlensäure-Überschuss stand (2). In Abb. 31 stellt die Abszisse die Mengen der zugesetzten Substanzen dar, ausgedrückt in den ihnen entsprechenden Cozymase-Mengen (Cozymase II). Dabei wurde mit dem von v. EULER und SCHLENK (1936) angegebenen Molekulargewicht der Cozymase gerechnet, nämlich 663 (vergl. MYRBÄCK, 1937, Seite 122). Aus Abb. 31 geht hervor, dass Cozymase die stärksten Effekte ausübt. Die nächst starke Wirkung hat Adenosin. Auch Adenylsäure übt in dem einen Versuch in kleinen Konzentrationen einen deutlichen Effekt aus. Ähnliche Ergebnisse wurden in anderen Versuchen erhalten. Man dürfte hieraus schliessen können, dass die stimulierende Wirkung auf die Oxydationsprozesse, die bei dem Zusatz von Cozymase in den in diesem Kapitel vorgelegten Versuchen gefunden wurde, auf einer spezifischen Wirkung der Cozymase beruht. Diese Wirkung dürfte in einer Steigerung der Dehydrierung einiger der in den Geweben vorhandenen Donator-Substanzen bestehen.

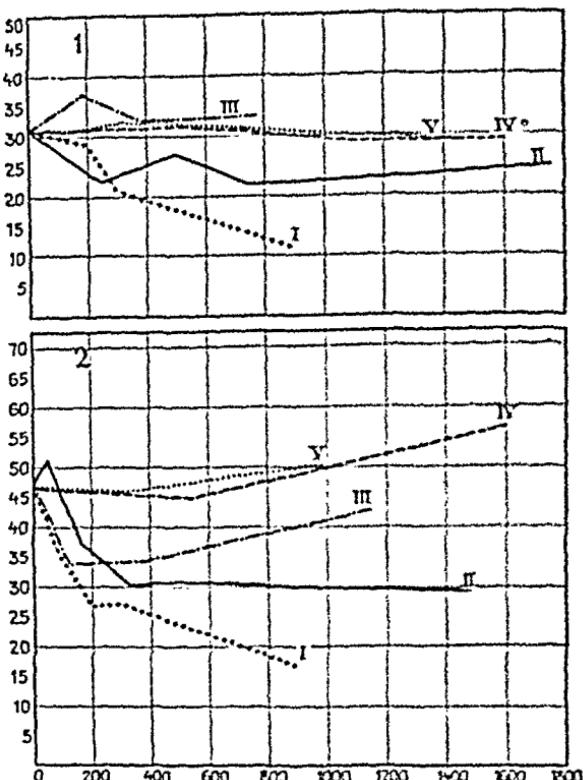


Abb. 31. Methylenblau-Versuche. Einwirkung von Cozymase I und von verschiedenen in der Cozymase enthaltenen Stoffen.

1. Normalversuch vom 11. 3. 1943.
2. Sauerstoffmangel und Kohlensäure-Überschuss, Versuch vom 22. 3. 1943.

- I. Cozymase I.
- II. Adenosin.
- III. Adenylsäure.
- IV. Nicotinsäure-Amid.
- V. Adenin.

Ordinate: Entfärbungszeiten in Minuten.

Abszisse: Zugesetzte Mengen Cozymase in  $\gamma$  (I), bzw. Mengen der anderen Substanzen (II bis V), umgerechnet in entsprechende Mengen  $\gamma$  Cozymase.

Es wäre denkbar, dass die Erhöhung der Oxydations-Intensität durch Adenosin und Adenylsäure auf verschiedenen Ursachen beruhen könnte. Eine Möglichkeit wäre, dass diese Substanzen von entsprechenden Aminasen zerstört würden, und dann eine Oxydation stattfinde. Adenin dürfte aber nicht in

den Geweben zerstört werden (vergl. OPPENHEIMER, 1936, Seite 557). Eine andere Erklärung wäre, dass von den genannten Substanzen die Zerstörung der Cozymase gehindert würde. MANN und QUASTEL (1941) zeigten, dass Nicotinsäure-Amid die Zerstörung der Cozymase durch eine im Gehirn vorhandene Nucleotidase hindert. Die Verfasser nehmen an, dass die Ursache hierfür wäre »a competition of nicotinamide with cozymase for the nucleotidase«. Die Autoren werfen die interessante Frage auf, ob nicht die therapeutischen Wirkungen des Nicotinsäureamids teilweise auf einem derartigen Effekt beruhen könnten.

HOLMBERG (1938) teilte Versuche mit, die dafür sprechen, dass Meerschweinchen-Leberextrakte Cozymase und einige Adenylsäuren oder ihre Spaltprodukte dehydrieren könnten. Es wäre daher nicht ganz ausgeschlossen, dass ein Teil der Steigerung, die in den hier vorgelegten Versuchen in der Oxydations-Intensität gefunden wurde, auf derartigen Prozessen beruhen könnte. Es ist aber aus quantitativen Gründen nicht wahrscheinlich, dass in den in diesem Kapitel weiter oben mitgeteilten Versuchen diese Verhältnisse eine grössere Rollen spielen könnten. HOLMBERG wendete in seinen Versuchen eine sehr kleine Konzentration von Thionin (25 γ) an. Ferner ist in den Versuchen von HOLMBERG das System nicht intakt. Die Haltbarkeit von beispielsweise Cozymase dürfte in derartigen Systemen schlecht sein (vergl. LENNERSTRAND, 1941).

Wie aus Abb. 29 hervorgeht, wird im Methylenblau-Versuch die Oxydations-Intensität der Gewebe von Tieren, die unter der Einwirkung eines Sauerstoffmangels und eines Kohlensäure-Überschusses standen, nach dem Zusatz von Cozymase wieder normal. Man darf daher vermuten, dass von den unter den fraglichen Bedingungen notwendigen Faktoren des enzymatischen Systemes es hauptsächlich die Cozymase ist, die geschädigt wird. Das Diaphorase-Flavoprotein dürfte dagegen unter den Bedingungen des Methylenblau-Versuches mindestens ausreichend intakt sein.

Dass der Zusatz von Cozymase keinen stimulierenden Effekt auf die Sauerstoffaufnahme, bestimmt nach der Warburg-Methode, ausübt, dürfte wahrscheinlich darauf beruhen, dass ausserdem noch ein anderer notwendiger Faktor geschädigt ist. Es ist nicht unwahrscheinlich, dass dieser Faktor in naher Beziehung zu dem Diaphorase-Flavoprotein-System oder auch

zu dem nicht sicher bekannten Verbindungsglied zwischen diesem System und dem Cytochrom-System steht. Um diese Fragen näher zu studieren, müssten *in vitro* verschiedene Flavinenzyme in ihrer Wirkung auf die herabgesetzten Geweboxydationen untersucht werden. Ein anderer gangbarer Weg wäre möglicherweise, Meerschweinchen verschiedene in der Cozymase und den gelben Fermenten enthaltene Substanzen zu injizieren, um vielleicht auf diese Weise einer Schädigung des fraglichen Fermentsystems entgegenzuwirken. Ein dritter Weg für die Fortsetzung der Arbeit wäre, mehr Donator-Substanzen zu untersuchen, als bisher in dieser Arbeit geprüft wurden. Diese Fragen mussten aber einer späteren Untersuchung vorbehalten werden.

Die in diesem Abschnitt nachgewiesene Schädigung der Cozymase in der Leber, zu der es unter der Einwirkung des Sauerstoffmangels und des Kohlensäure-Überschusses kommt, kann möglicherweise zum Verständnis des früher besprochenen Verhaltens beitragen, dass nämlich die Herabsetzung der Geweboxydationen in der Leber scheinbar leichter erfolgt, wenn Sauerstoffmangel und Kohlensäure-Überschuss gleichzeitig einwirken. Aus Untersuchungen von u.a. v. EULER und seinen Mitarbeitern (1938) geht hervor, dass die Dihydrocozymase in saurerem Milieu weniger stabil ist, ein Verhalten, das der Bestimmung der oxydierten und der reduzierten Form der Cozymase zu Grunde liegt. Wie oben auseinandergesetzt, sprechen bestimmte experimentelle Befunde dafür, dass bei schwerem akutem Sauerstoffmangel im Organismus eine Verschiebung des Verhältnisses Cozymase: Dihydrocozymase in der Richtung erfolgt, dass die Dihydrocozymase überwiegt. Wenn jetzt gleichzeitig ein Kohlensäure-Überschuss besteht, so wäre es denkbar, dass infolgedessen der Zerfall der Dihydrocozymase vermehrt ist. Diese mögliche Erklärung wird mit allem Vorbehalt angeführt, da die Stabilitätsverhältnisse der Cozymase *in vivo* nicht bekannt sein dürften.

In der Nachperiode nach einem langdauernden Asthma können die Versuchstiere matt aussehen, ihre Körper- und Hauttemperatur kann lange Zeit erniedrigt sein (siehe Abb. 11). Im Hinblick auf die oben beschriebenen Veränderungen im Cozymase-

System, wäre es nicht ausgeschlossen, dass dieser Zustand der Versuchstiere durch eine Störung der intermediären enzymatischen Prozesse verursacht wäre.

Wenn die Versuchstiere unter der Einwirkung des Sauerstoffmangels und des Kohlensäure-Überschusses stehen, sind meist die Veränderungen der Geweboxydationen in der Leber am deutlichsten. LENNERSTRAND (1941) zeigte, dass die Stabilität der Cozymase auf einem intakten glykolytischen System beruht. Wie O'NEILL, BING Moy und MANWARING (1925) zeigten, verschwindet im anaphylaktischen Schock das Glykogen fast vollständig aus der Leber. Es dürfte nicht ausgeschlossen sein, dass dies die Glykolyse herabsetzen und dadurch zu einer Verminderung der Stabilität der Cozymase beitragen könnte. In Verfolgung dieses Gedankenganges wäre es interessant, die Veränderungen der Geweboxydationen im experimentellen Asthma zu prüfen, nachdem den Versuchstieren Zucker zugeführt wurde.

*Cocarboxylase:* In einigen Versuchen wurde (mit der Warburg- wie mit der Methylenblau-Methode) der Einfluss der Cocarboxylase auf die herabgesetzte Gewebsatmung im experimentellen Asthma geprüft. Dabei konnte kein stimulierender Effekt konstatiert werden. Die zugesetzten Mengen gingen bis zu 1 mg. Wie aus Tabelle 4 hervorgeht, braucht bei Sauerstoffmangel und Kohlensäure-Überschuss keine deutliche Erhöhung des Brenztraubensäure-Gehaltes in vivo zu erfolgen, obgleich die Geweboxydationen herabgesetzt waren. Dies ganze Verhalten spricht möglicherweise dafür, dass keine Störung in den Cocarboxylase-Funktionen vorliegt.

### Zusammenfassung.

*Die herabgesetzten Geweboxydationen in der Leber von Versuchstieren, die unter der Einwirkung eines experimentellen Asthmas oder eines Sauerstoffmangels und Kohlensäure-Überschusses standen, können im Warburg- wie im Methylenblau-Versuch durch den Zusatz von Succinat erhöht werden. Hieraus dürfte der Schluss zu ziehen sein, dass das Cytochrom-System inclusive des STRAUB'schen SC-Faktors *in vitro* intakt ist. Die Versuche stützen nicht die Auffassung, dass die Wirkung des*

*Succinates eine C<sub>4</sub>-Dicarbonsäure-Katalyse nach SZENT-GYÖRGYI darstellt.*

*Der Cozymase-Gehalt des Lebergewebes von Versuchstieren, die ein experimentelles Asthma hatten oder unter der gleichzeitigen Einwirkung eines Sauerstoffmangels und Kohlensäure-Überschusses standen, ist herabgesetzt. Die verminderten Gewebeoxydationen bei diesen Zuständen können durch den Zusatz von Cozymase im Methylenblau-Versuch, aber nicht im Warburg-Versuch erhöht werden.*

Bei dem Zusatz von Cocarboxylase wurde kein Effekt auf die herabgesetzten Gewebeoxydationen gefunden.

## KAP. VIII.

### Schlussbemerkungen und Zusammenfassung.

Die Untersuchungen dieser Arbeit gingen davon aus, dass die Sauerstoffaufnahme bei verschiedenen Formen der Stenoseatmung nach den bisherigen Angaben der Literatur nicht in einheitlicher Richtung verändert wäre. Die eigenen Versuchsergebnisse, die mit einer Form der Stenoseatmung gewonnen wurden, die klare und übersichtliche Versuchsbedingungen im Tierexperiment gestattet, nämlich dem experimentellen Asthma, ausgelöst durch Histamin-Inhalation oder auf anaphylaktischem Wege, liessen aber erkennen, dass die scheinbaren Widersprüche der bisherigen Befunde durch die Differenzen im Schweregrad der Atmungsbehinderung und durch den verschiedenen Zeitpunkt der Untersuchung erklärt werden können. Im leichten experimentellen Asthma des Meerschweinchens steigen die Lungenventilation, die Sauerstoffaufnahme und der respiratorische Quotient an. Bei schwerem Asthma dagegen nehmen die Lungenventilation, die Sauerstoffaufnahme und der respiratorische Quotient ab. Die in der Nachperiode beobachteten Veränderungen dieser Grössen hängen ebenfalls allein von dem Schweregrad des überstandenen Asthmas ab: Nach einem leichten Asthma sinken die Lungenventilation und die Sauerstoffaufnahme wieder zur Norm ab, nach einem schweren Asthma steigen die Lungenventilation und die Sauerstoffaufnahme weit über die Norm an. Während eines schweren Asthmas ist die Sauerstoffsättigung des arteriellen Blutes, zum Teil sehr erheblich, vermindert; gleichzeitig ist der Milchsäure-Gehalt des arteriellen Blutes erhöht. Diese Befunde im Zusammenhang mit dem Verhalten des respiratorischen Quotienten lassen erkennen, dass der asthmatische Zustand der Versuchstiere durch

Sauerstoffmangel und einen gleichzeitig bestehenden Kohlensäure-Überschuss im Organismus gekennzeichnet ist. Als Ursache dieser Veränderungen dürfen wir die Erschwerung des Gasaustausches in der asthmatischen Lunge ansprechen.

Es lag nahe, die Analyse des Verhaltens der oxydativen Prozesse im Asthma bezw. unter der Stenoseatmung in der Richtung weiterzuführen, dass die Geweboxydationen selbst mit den hierfür geeigneten Methoden (Warburg- und Methylenblau-Versuch) untersucht wurden, dies um so mehr, als ABDERHALDEN und WERTHEIMER auf Grund ihrer Befunde die Herabsetzung der Gewebsatmung im anaphylaktischen Schock als für diesen Zustand spezifisch ansahen. In meinen Untersuchungen an den Geweben von Tieren, die im Histamin-Asthma starben oder getötet wurden, zeigte sich, dass die oxydativen Prozesse in den meisten untersuchten Geweben mehr oder weniger herabgesetzt waren. Die stärkste Erniedrigung der Gewebsatmung liess stets die Leber erkennen, während die Nierenrinde in ihrer Atmungsgrösse unverändert blieb. Eine bis in alle Einzelheiten gleichartige Herabsetzung der Geweboxydationen findet man in den Organen von Versuchstieren, die vor ihrer Tötung der Einwirkung eines Sauerstoffmangels und gleichzeitigen Kohlensäure-Überschusses ausgesetzt wurden. Da wir, wie oben bemerkt, während des asthmatischen Zustandes im Gesamtorganismus mit dem Bestehen eines Sauerstoffmangels und Kohlensäure-Überschusses rechnen dürfen, kann für die Entstehung der Veränderungen der Gewebsatmung im Asthma der Schluss gezogen werden, dass sie ebenfalls durch den Sauerstoffmangel und gleichzeitigen Kohlensäure-Überschuss hervorgerufen werden. Damit zeigte sich auch, dass die Herabsetzung der Geweboxydationen im anaphylaktischen Schock nicht, wie früher von ABDERHALDEN und WERTHEIMER angenommen, für diesen Zustand spezifisch sein dürfte. Vielmehr haben wir ganz allgemein bei jedem Zustand, der mit einem Sauerstoffmangel und Kohlensäure-Überschuss im Organismus einhergeht, eine Herabsetzung der Gewebsatmung zu erwarten.

Versucht man die Entstehungsbedingungen der Herabsetzung der Geweboxydationen weiter zu analysieren, so muss man sich fragen, ob der ursächlich so bedeutungsvolle Sauerstoffmangel

und Kohlensäure-Überschuss zu einer generellen Schädigung der enzymatischen Prozesse in den Zellen führt, oder ob nur einzelne Glieder des Atmungssystems geschädigt sind. Die in dieser Richtung angestellten Untersuchungen zeigen, dass der Zusatz von Succinat zu Lebergewebe im Warburg-Versuch die erniedrigte Gewebsatmung erhöht. Hieraus ist zu schliessen, dass das Cytochrom-System *in vitro* intakt ist. Setzt man im Methylenblau-Versuch der Leber, deren oxydative Prozesse durch ein Histamin-Asthma oder die Einwirkung des Sauerstoffmangels oder Kohlensäure-Überschusses herabgesetzt sind, Cozymase zu, so steigt die Gewebsatmung erheblich an, und erreicht die gleiche Grösse, wie man sie nach dem Zusatz von Cozymase zu normalem Lebergewebe beobachtet. Wie sich ferner zeigte, ist der Cozymase-Gehalt der Leber von Versuchstieren, die im Histamin-Asthma starben bzw. getötet wurden oder die unter der Einwirkung eines Sauerstoffmangels und Kohlensäure-Überschusses standen, deutlich vermindert. Aus diesen Versuchsreihen ist zu schliessen, dass eine Schädigung der Funktionsabläufe vorliegt, in die die Cozymase normalerweise eingreifen muss. Da bekanntlich die Cozymase für verschiedene Oxydo-Reduktions-Prozesse in den Zellen von wesentlicher Bedeutung ist, liegt es nahe, diese Befunde zur Erklärung der in dieser Arbeit nachgewiesenen Herabsetzung der Gewebsatmung im experimentellen Asthma heranzuziehen, wenn auch betont werden muss, dass die Schädigung des Cozymase-Systems nicht die alleinige Ursache dieser Veränderungen sein kann.

Die Entstehungsbedingungen der Herabsetzung der Gewebsoxydationen im experimentellen Asthma mögen noch in manchen Punkten einer eingehenderen Klärung bedürfen. Wesentlich scheint aber für jede weitere Analyse das hier festgestellte Verhalten zu sein: jede schwere Stenoseatmung verändert schon an und für sich die oxydativen Prozesse in den Geweben.

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Tabelle 7.

Datum	Gewicht, Geschlecht der Ver- suchstiere	Art und Dauer der Versuche	Senkung der Rektal- temp. Todes- ursache <sup>1)</sup>	Wärburg-Versuche (mm O <sub>2</sub> -Aufnahme pro 1/2 St.)				
				Leber.	Ober- schenkel musk.	Zwerch- fell	Herz- musk.	Niere
11.11.1941	650 ♀	N.	76; 86	123; 122	59; 63	95; 90	52; 54	25
15.11.1941	550 ♂	N.	68; 91	129; 122	54; 57	103; 99	49	38
17.11.1941	500 ♂	N.	86; 78	140; 132	105; 101	131; 171	30	31
18.11.1941	325 ♂	N.	47; 88	180; 166	66	72; 113	22	71
19.11.1941	620 ♂	H <sub>2</sub> ?	X	73; 85	155; 144	46	113; 111	35; 41
21.11.1941	470 ♂	H <sub>2</sub> ?	2°, 7	28; 26	39; 42	32	110; 115	50
22.11.1941	460 ♂	H <sub>2</sub> ?	1°, 35/	138; 142	195; 193	52	76; 87	27
24.11.1941	620 ♂	H <sub>2</sub> ?	N.	104; 108	88; 86	51; 53	122; 123	26
25.11.1941	475 ♂	H <sub>2</sub> ?	0°, 3 †	27; 32	176; 185	33; 37	21	34
9.1.1942	400 ♂	O <sub>2</sub> Mangel	1°, t, 6 X	124; 141	110; 118	46; 48	38; 37	97
10.1.1942	400 ♂	O <sub>2</sub> Mangel	{CO <sub>2</sub> -Überschuss	1°, 2 X	26; 21	147; 143	32; 34	114; 111
12.1.1942	380 ♂	O <sub>2</sub> Mangel	{CO <sub>2</sub> -Überschuss	2° X	103; 118	150; 144	86; 104	105; 109
13.1.1942	300 ♂	O <sub>2</sub> Mangel	{CO <sub>2</sub> -Überschuss	2°, 2 X	47; 51	46; 48	33; 36	25; 29
14.1.1942	300 ♂	N.	{CO <sub>2</sub> -Überschuss	N.	115; 131	125; 102	72; 76	105; 109
16.1.1942	380 ♂	O <sub>2</sub> Mangel	{CO <sub>2</sub> -Überschuss	2° X	138; 125	109; 109	36; 40	114; 111
29.1.1942	460 ♂	N.	{CO <sub>2</sub> -Überschuss	N.	77; 81	111; 113	129; 106	112; 107
2.2.1942	415 ♂	O <sub>2</sub> Mangel	{CO <sub>2</sub> -Überschuss	1°, 4 X	52; 65	91; 85	34; 36	94
3.2.1942	400 ♂	O <sub>2</sub> Mangel	{CO <sub>2</sub> -Überschuss	0,7 X	80; 108	88; 86	73; 66	111; 111
4.2.1942	400 ♂	O <sub>2</sub> Mangel	{CO <sub>2</sub> -Überschuss	N.	140; 158	162; 158	62; 60	183; 135
6.2.1942	380 ♂	O <sub>2</sub> Mangel	{CO <sub>2</sub> -Überschuss	N.	32; 37	97; 99	34; 36	132; 120
11.2.1942	400 ♂	O <sub>2</sub> Mangel	{CO <sub>2</sub> -Überschuss	N.	105; 116	134; 146	86	161; 120
16.2.1942	400 ♂	O <sub>2</sub> Mangel	{CO <sub>2</sub> -Überschuss	N.	1°, 4 †	42; 40	131; 135	163
18.2.1942	420 ♂	O <sub>2</sub> Mangel	{CO <sub>2</sub> -Überschuss	N.	122	196	63	84
19.2.1942	390 ♂	O <sub>2</sub> Mangel	{CO <sub>2</sub> -Überschuss	N.	0°, 5 †	40	81	125
22.2.1942	390 ♂	O <sub>2</sub> Mangel	{CO <sub>2</sub> -Überschuss	N.	0°, 6 X	104	,115	124

) † = getötet; X = gestorben      <sup>2)</sup> H<sub>2</sub>. Histamin-Asthma, Grad 2.

Tabelle 7.

Datum	Methylenblau-Versuche (Entfütterungszeit in Minuten)						Zugesetzte Menge Methylenblau in γ	$\text{O}_2$ -Gehalt	$\text{CO}_2$ -Gehalt	Gasgemische im Apparate bei Versuchsende
	Leber	Oberschenkel-musk.	Zwerchfell	Herz-musk.	Niere	Gehirn				
11.11.1941	22; 21	15; 16	23; 23		25; 26	22; 23	26; 25	19; 19	1000/5cc	
15.11.1941	29; 24	20; 21	21; 20	25; 26	20; 18	31	35; 36	16; 20	2	
17.11.1941	24; 26	20; 20	26; 27		16; 16	32; 37	62; 72	20; 20	2	
18.11.1941	28; 28	20; 20	28		31; 30	41; 37	64	20; 20	2	
19.11.1941	26; 26	23; 23			18; 18	40; 42	26	20; 20	2	
21.11.1941	122; 133	40; 55	66		30; 26	49; 35	> 160	54; 57	2	
22.11.1941	22; 22	18	25	20; 20	23; 24					
24.11.1941	37; 35	24; 25		39	23; 21					
25.11.1941	57; 60	21; 19	85	78	19; 19					
9.1.1942										
10.1.1942										
12.1.1942										
13.1.1942										
14.1.1942										
16.1.1942										
29.1.1942	24; 26	21; 23	15; 20	16; 18	91; 97	22	28; 26	21; 21	4,02	3,76
2.2.1942	41; 45	48; 51							15,03	3,78
3.2.1942	26; 26	32; 34		27; 27					4,00	4,30
4.2.1942	26; 23	25; 24							—	—
6.2.1942	52; 54	31; 30	44		57; 67					
11.2.1942	27; 29	24; 24	28; 23	25; 23	22					
16.2.1942	70; 74	30			78					
18.2.1942	37				27					
19.2.1942	59; 64				30; 29					
23.2.1942	55; 56				70	74	25	4,36	5,39	4,10
					24; 26	41	53	4,37		

0,37%

Tabelle 7.

Datum	Gewicht Geschlecht, der Ver- suchstiere	Art und Dauer der Versuche	Senkung d. Rektal- temp.	Todesur- sache † = gestor- ben X = getötet	Warburg-Versuche	
					Leber	Ober- schenkel- musk.
14. 6. 1942	640 ♂	Normal			118; 193	193; 198
15. 6. 1942	740 ♂	,			109	217; 291
26. 6. 1942	700 ♂	H. 2 <sup>b</sup> 30' <sup>a</sup> )	1°,8	X	21	163
29. 6. 1942	820 ♂	H. 1 <sup>b</sup> 30'	1°,8	X	22	182
1. 7. 1942	870 ♂	Normal			154	203
2. 7. 1942	820 ♂	H. 20'	0°,5	†	118	171
3. 7. 1942	660 ♂	H. 1 <sup>b</sup> 50'	0°,2	X	37	250
16. 7. 1942	850 ♂	H. 2 <sup>b</sup>	3°	X	24	117
22. 7. 1942	520 ♂	H. 2 <sup>b</sup>	1°,5	†	27	129
4. 11. 1942	320 ♂	H. 20'	0°,6	†	26	51
5. 11. 1942	400 ♂	Normal			62	139
14. 12. 1942	380 ♂	,			85	157
15. 12. 1942	350 ♂	Hunger 48 <sup>b</sup>			41	87
21. 12. 1942	430 ♂	H. 1 <sup>b</sup> 7'	2°,4	X	33	113
23. 12. 1942	370 ♂	H. 1 <sup>b</sup> 30'	1°	X	34	86
29. 12. 1942	420 ♂	H. 1 <sup>b</sup> 30'	1°,5	X	28	139
7. 1. 1943	460 ♂	Normal			107; 102	—
10. 1. 1943	450 ♂	H. 40'	0°,5	†	31; 39	—
13. 1. 1943	480 ♂	H. 50'	1°	X	39	—
15. 1. 1943	400 ♂	Normal			63	—
26. 1. 1943	410 ♂	,			133; 131	—
28. 1. 1943	430 ♂	H. 5'		†	103; 103	—
29. 1. 1943	540 ♂	H. 1 <sup>b</sup> 5'			81; 91	—
1. 2. 1943	530 ♂	Normal			101; 97	—
2. 2. 1943	530 ♂	{O <sub>2</sub> -Mangel  CO <sub>2</sub> -Überschuss	1°,1	X	62; 71	—
8. 2. 1943	490 ♂	Normal			79; 81	—
9. 2. 1943	410 ♂	,			122; 120	—
10. 2. 1943	450 ♂	Serum	0		101	—
22. 2. 1943	430 ♂	{O <sub>2</sub> -Mangel  O <sub>2</sub> -Überschuss	0°,3	†	38	132
23. 2. 1943	550 ♂	Normal			91	169
26. 2. 1943	380 ♂	O <sub>2</sub> -Mangel	1°,8	X	101	143
3. 3. 1943	530 ♂	{O <sub>2</sub> -Mangel  CO <sub>2</sub> -Überschuss	0°,3	X	57	—
11. 3. 1943	460 ♂	Normal			126; 130	181; 164
22. 3. 1943	530 ♂	{O <sub>2</sub> -Mangel  CO <sub>2</sub> -Überschuss	1°,3	X	99; 69	—

<sup>a</sup> Histamin-Asthma.

Tabelle 7.

Methylenblau-Versuche				Zugesetzte Menge von Methylen- blau in γ	Gasgemisch im Apparat bei Ver- suchsende		Grad des Asthmas
Leber	Oberschenkel musk.	Herz- musk.	Niere		O <sub>2</sub> %	CO <sub>2</sub> %	
27; 20	30; 33			1000/1,3 cc			
24; 24	24; 29			»			
72	30			»			3—2—3
80; 71; 97; 88	36; 27; 26; 35			»			5—4—3
27; 23; 27; 29	22; 19; 27; 21			»			
39; 36; 44; 39	28; 21; 20; 23			»			5
42; 48; 50; 41	36; 29; 35			»			3—4
40; 49	40; 36			»			3—4—3
71; 61	30; 33			»			3—4—3
52	24			»			1—2
23	33			»			
—	—			»			
23; 24	37; 30	22; 14		»			
—	—			»			3
—	—			»			3
—	—			»			3—5
29	33	23	39	»			
48	33	50	53	»			2—3
34	48	34	31	»			3—4
30; 25; 31	41; 44; 40	36; 33; 44	34; 37; 37	»			
37; 33; 35	40; 44; 38	44; 49; 55	34; 32; 33	»			1—3
49; 50; 47	47; 43; 51	47; 48; 55	45; 33; 35	»			3—2—3
34; 36; 33	51; 43; 46	45; 52; 54	32; 31; 30	»			
51; 54; 56	48; 47; 54	75; 64; 69	42; 42; 34	»	3,61	2,80	
36; 37; 38	45; 42; 47	45	43; 36; 41	»			
40; 36; 33	43; 41; 43	54	35; 34; 30	»			
34; 35; 35	52; 51; 45		41; 32; 27	»			
83; 63	56; 50; 52		54; 49	»			
45; 43; 40		48	50; 52	»			
33; 35; 35	54; 50		34; 41; 45	»	2,71	0,32	
71; 60; 70	61; 55	43		»	3,69	2,33	
30; 34; 28	55; 56			»			
55; 42; 50	42; 37	74		»			